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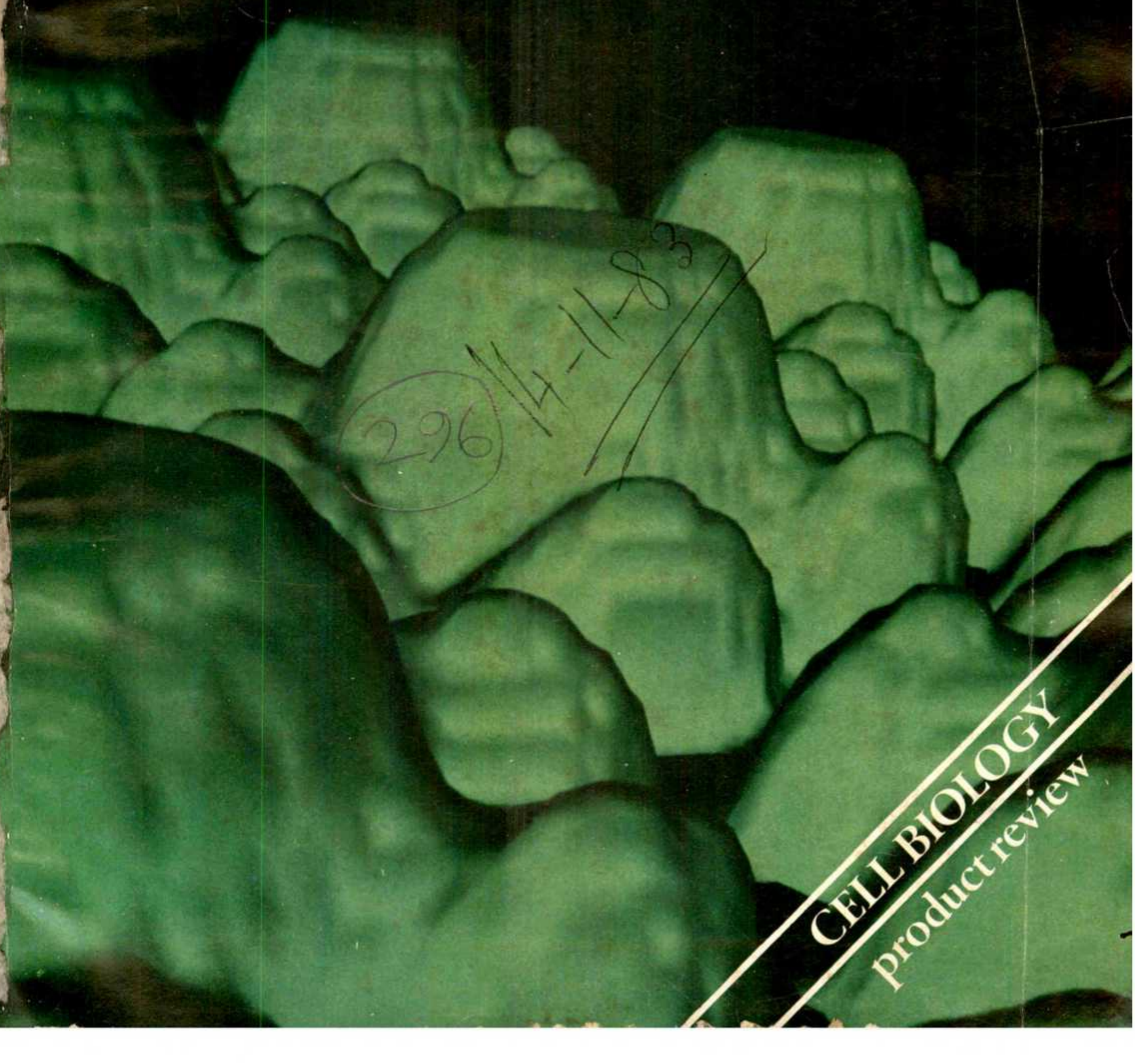

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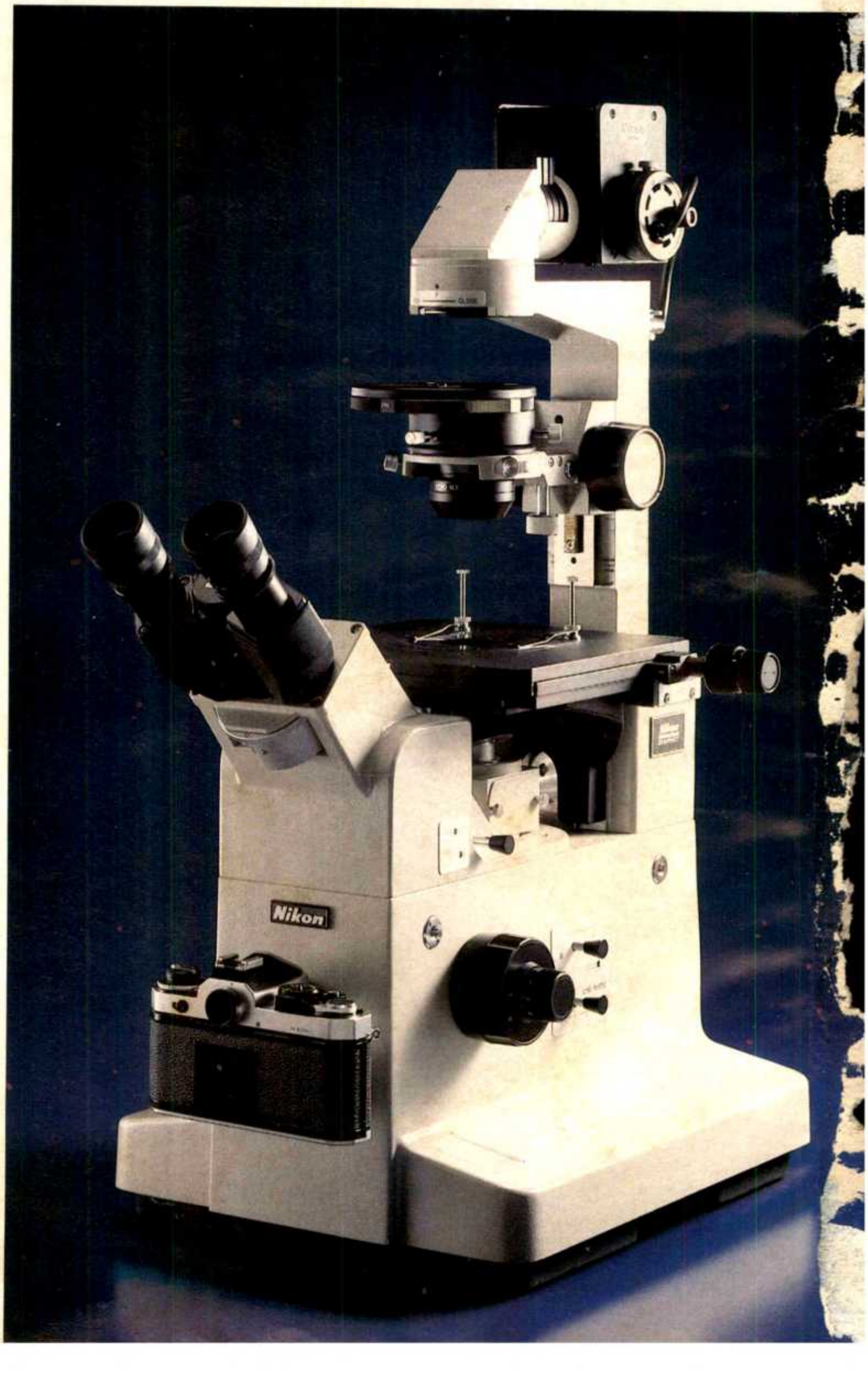

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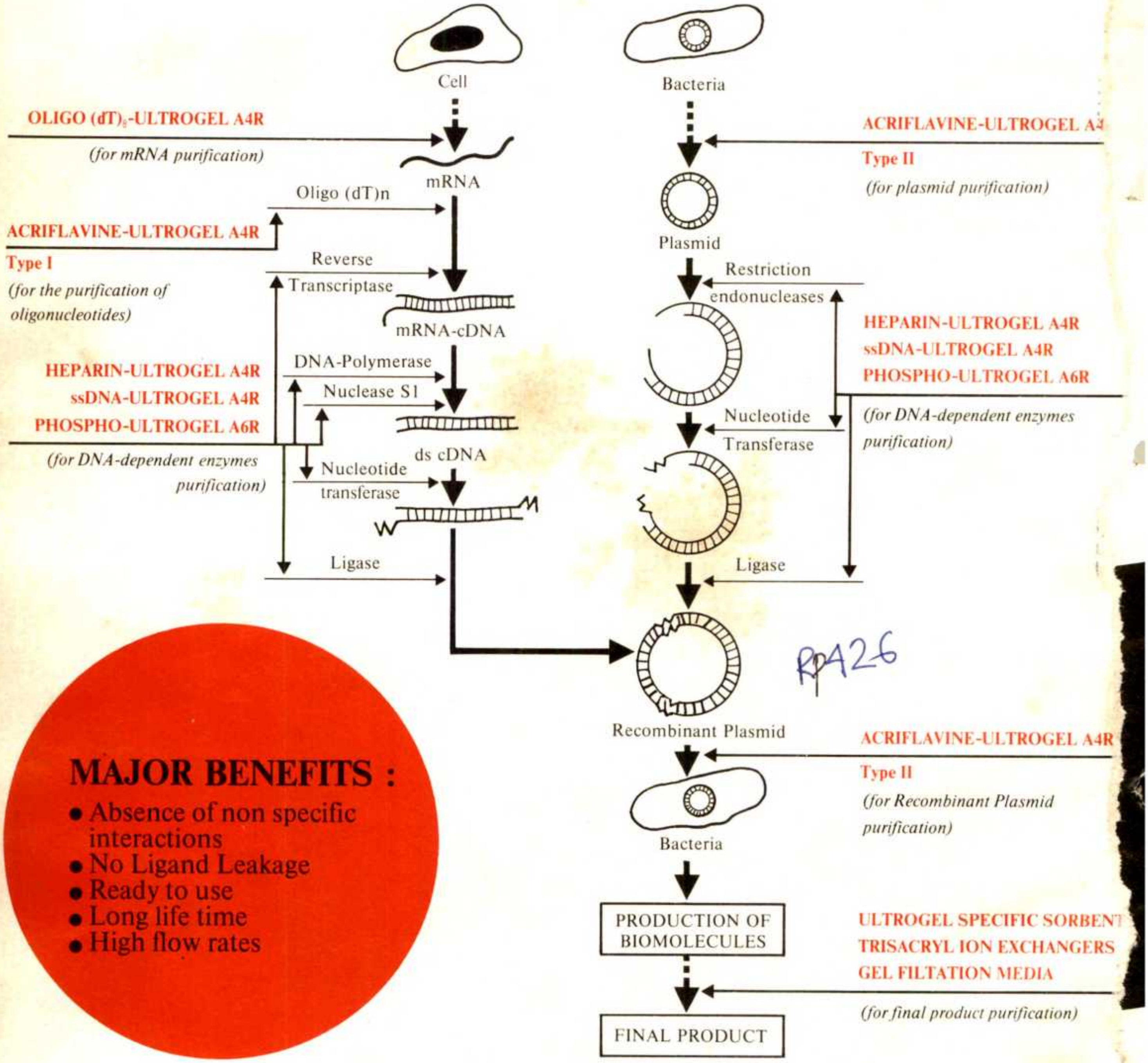

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Marvin Harris: Let's go back again to the possibility that these phobias are genetically programmed – which I'm willing to grant. The overwhelming bulk of the socially conditioned response repertories of different human societies consists, by your own admission, of culturally determined rather than genetically determined traits. Then it seems to me that when one offers a cogent culturological explanation of these
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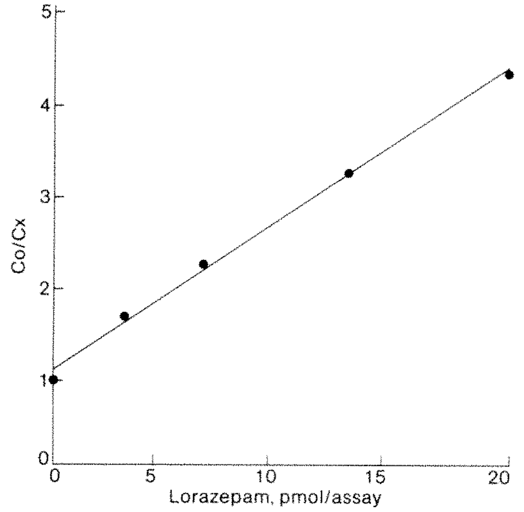

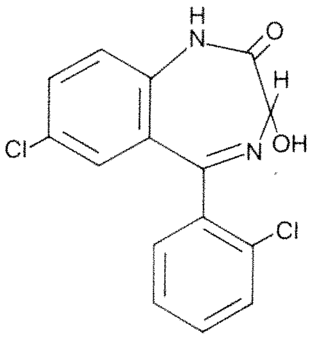

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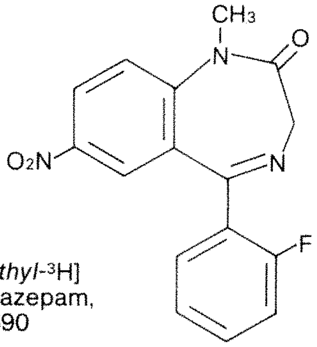

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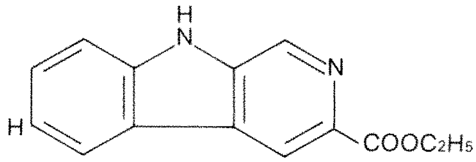

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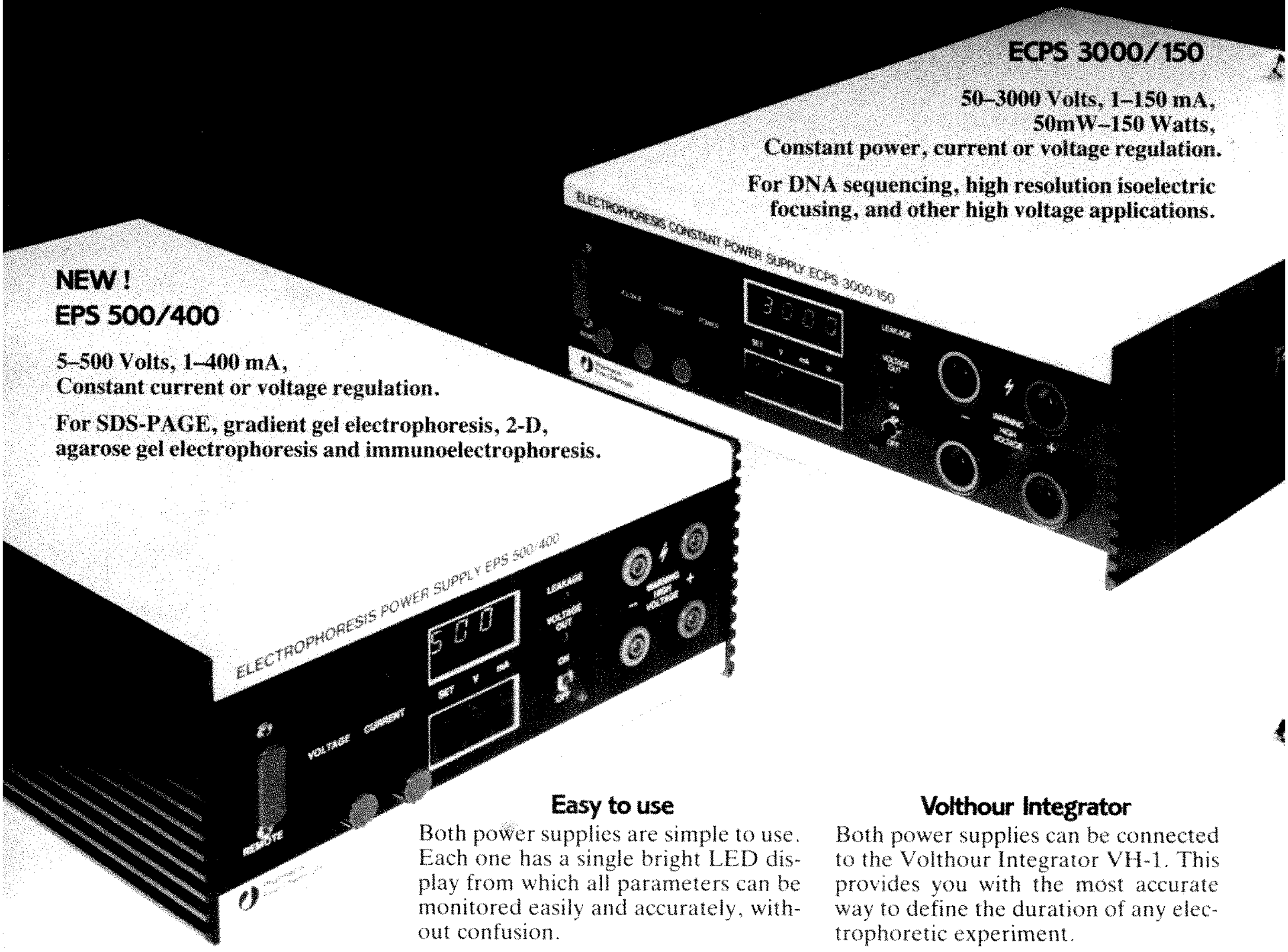
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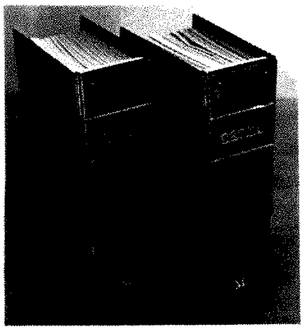

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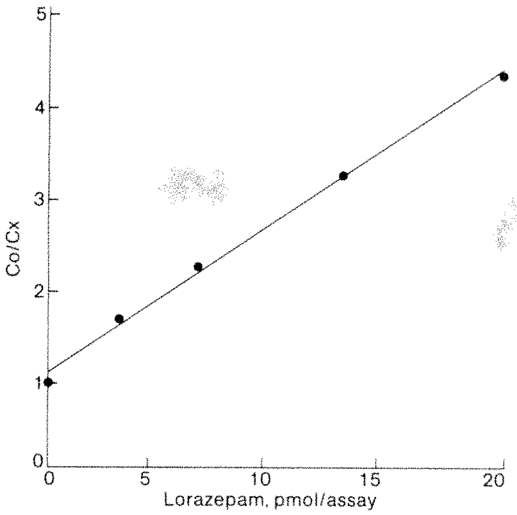

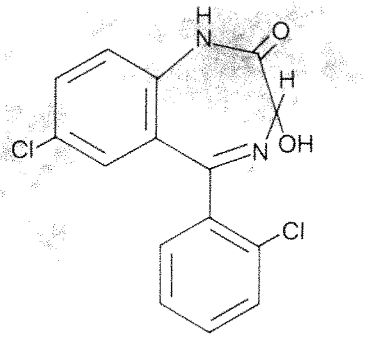

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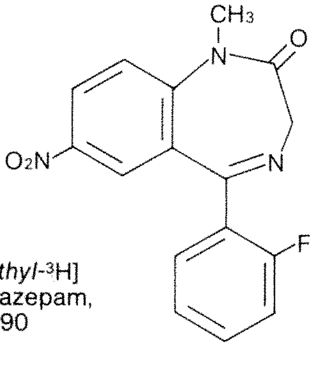

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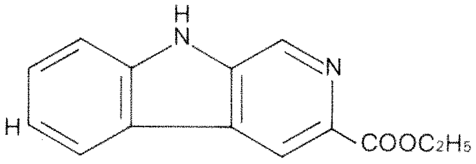

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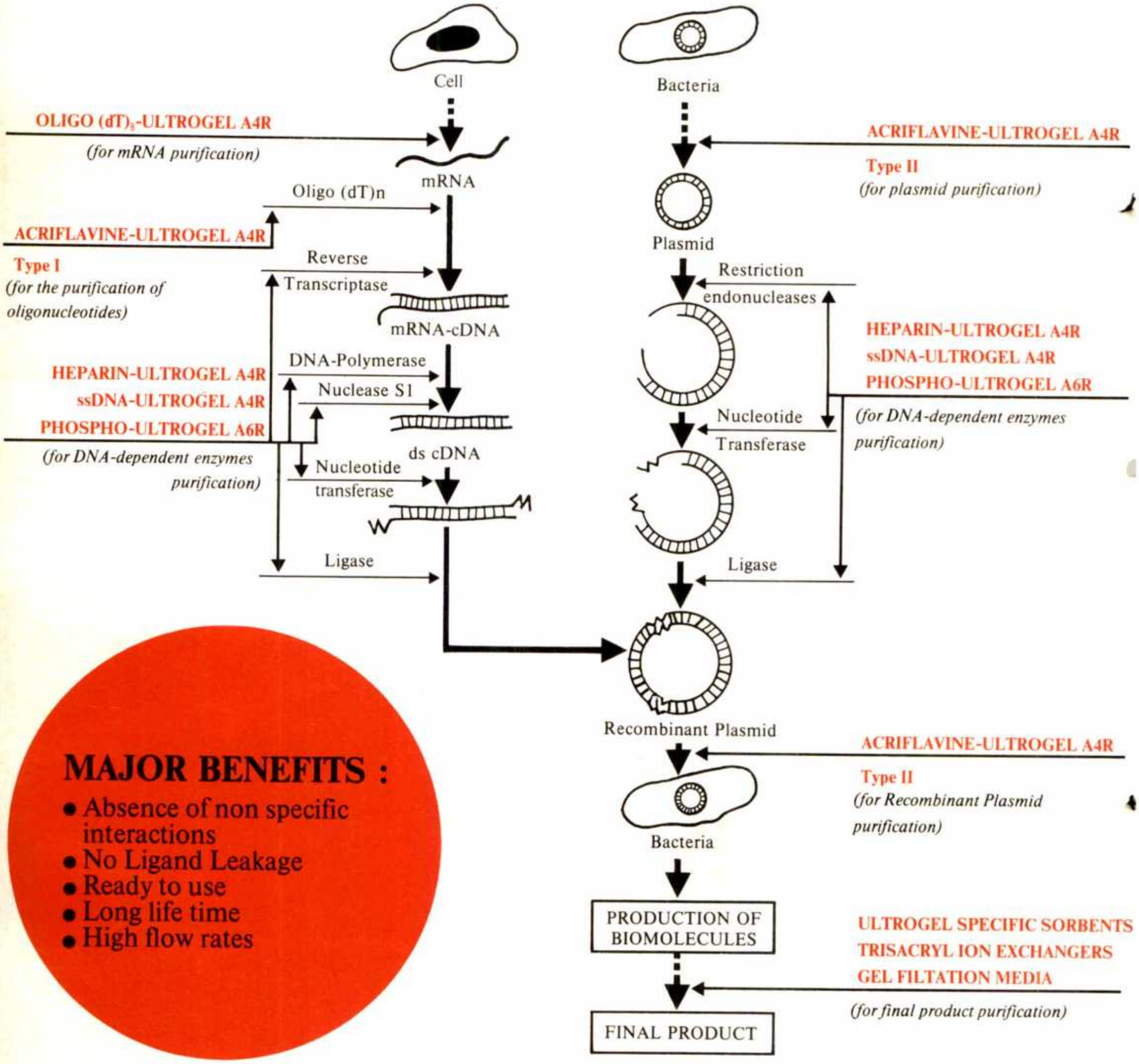

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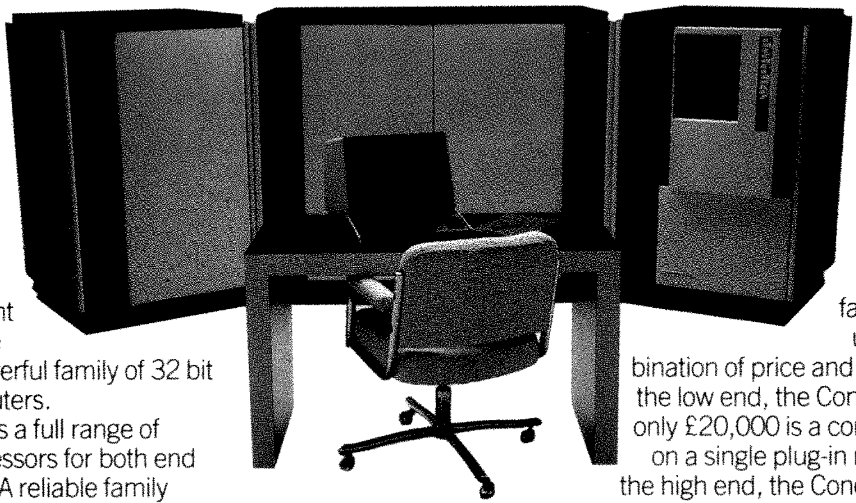

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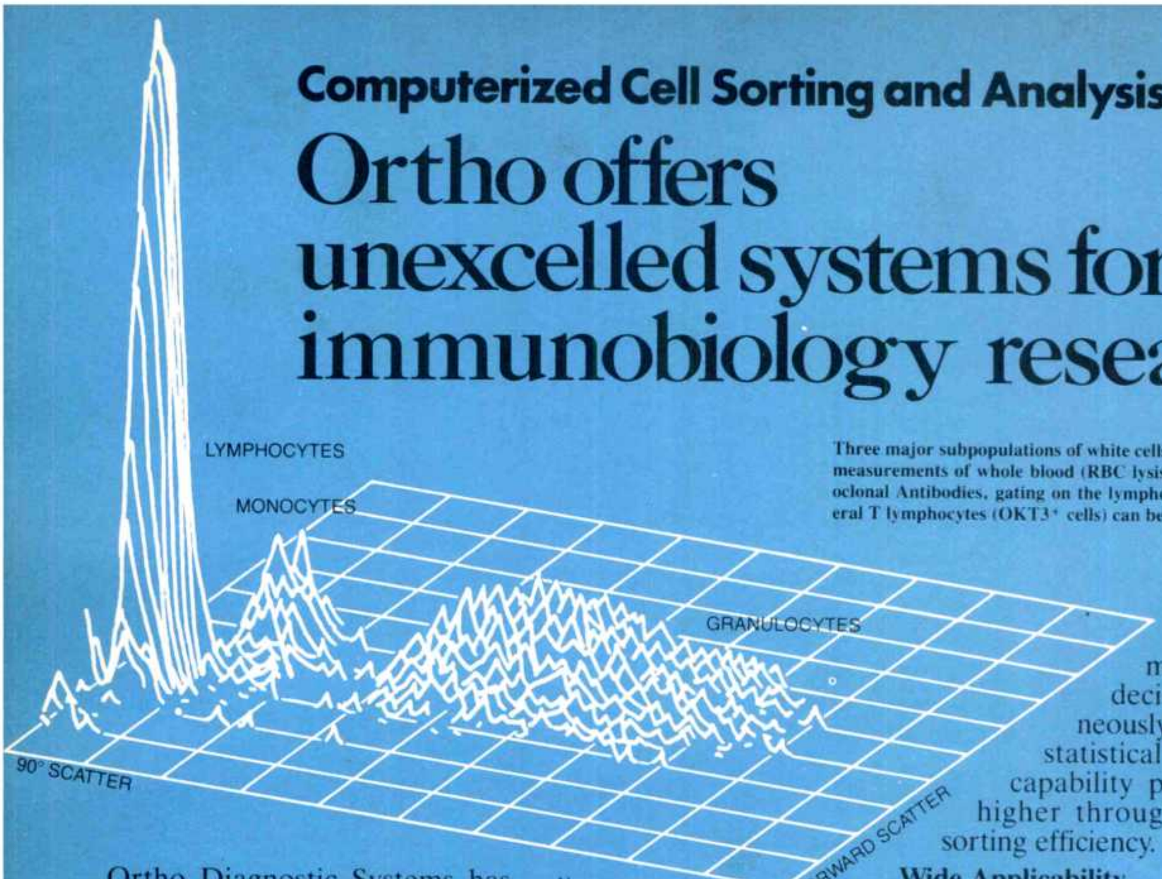

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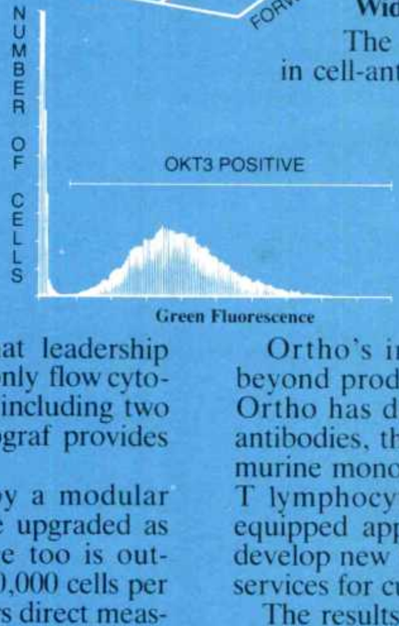

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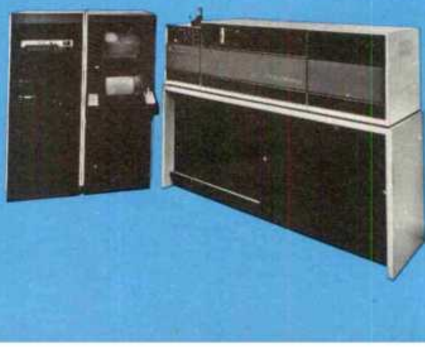

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- **Non-penetrating plasma membrane isolation reagent**[1]

TRK.696

[^3H] **IDNPAP**

Isethionyl-3 (*N*-2,4-dinitro-[3,5-^3H] phenyl) aminopropioimidate leaves the net charge on the protein unaltered. Consequently, the labelled and unlabelled proteins will have the same characteristic pattern when isolated by electrophoretic methods[2].

- **A bi-functional protein cross-linking and labelling reagent**

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Dimethyl[1, 8-^{14}C] **suberimidate**

$CH_3O\underset{\underset{NH}{\|}}{\overset{*}{C}}(CH_2)_6\underset{\underset{NH}{\|}}{\overset{*}{C}}OCH_3.2HCl$

This reagent has many applications as a bi-functional reagent capable of cross-linking closely neighbouring proteins. A list of references is available on request.

- **A versatile and selective reducing agent**

TRK.708

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CFA.700

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1. DENNY, J.B. and ROBERTS, R.M., *Journal of Biological Chemistry*, **257**, pp. 2460-2468, 1982
2. WOFSY, L. and SINGER, S.J., *Biochemistry*, **2**, pp. 104-106, 1963
3. JENTOFF, N. and DEARBORN, D.G., *Analytical Biochemistry*, **106**, pp. 186-190, 1980

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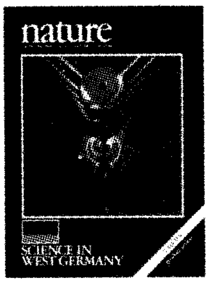

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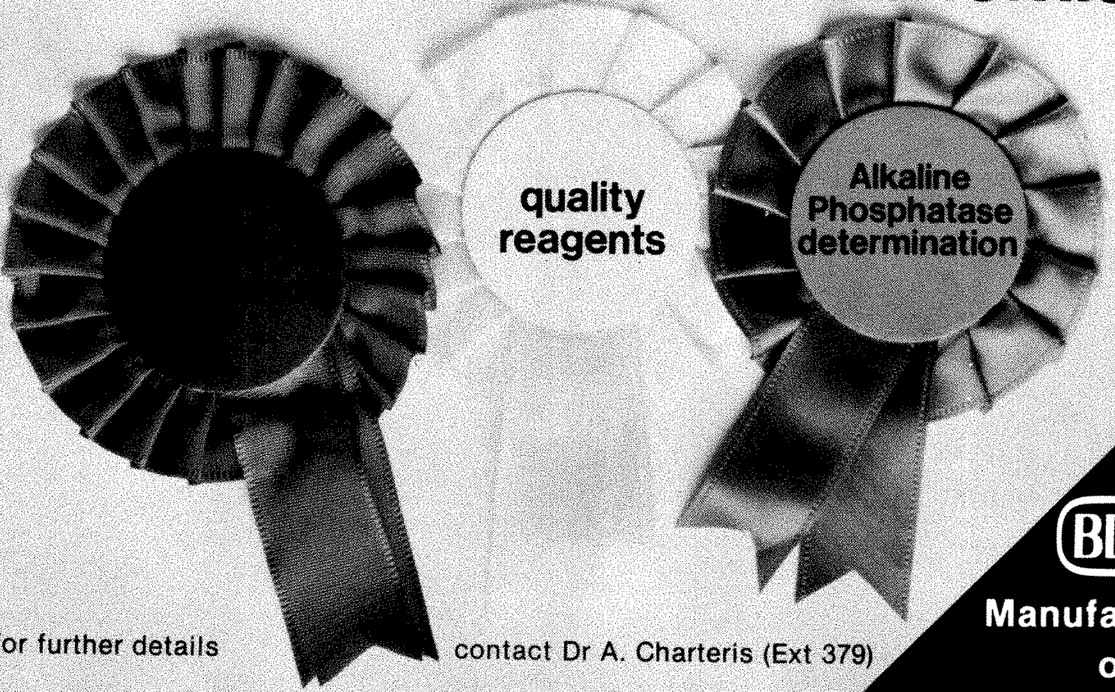

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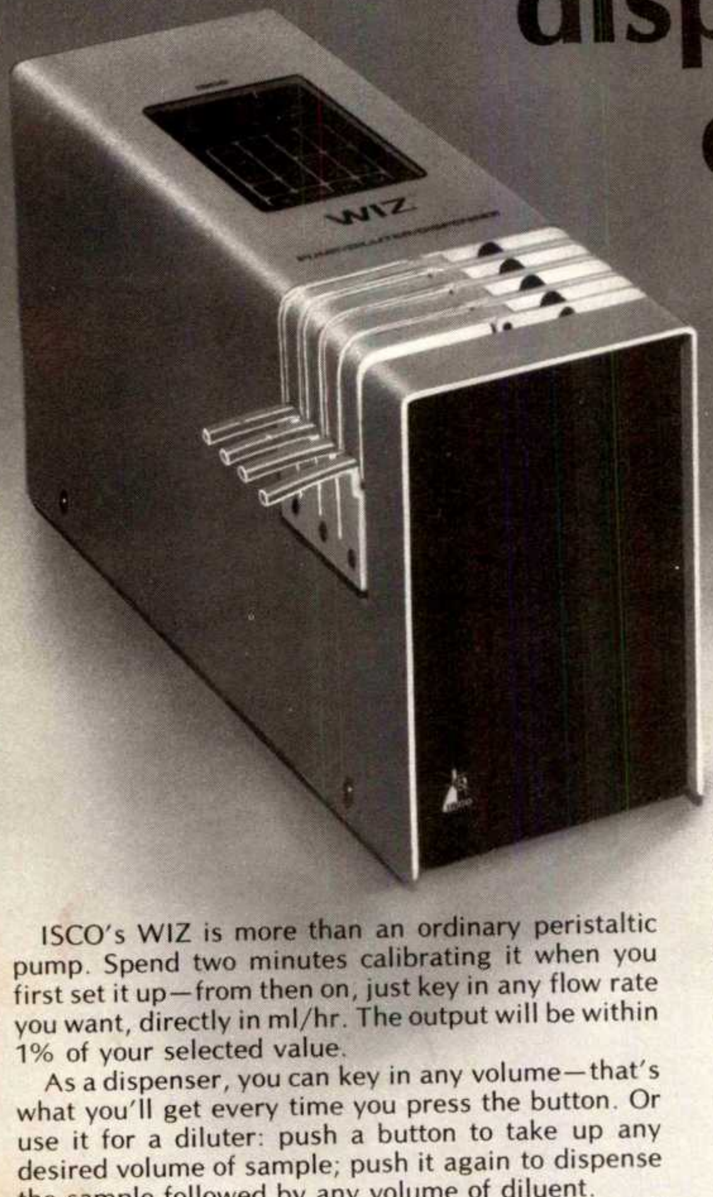

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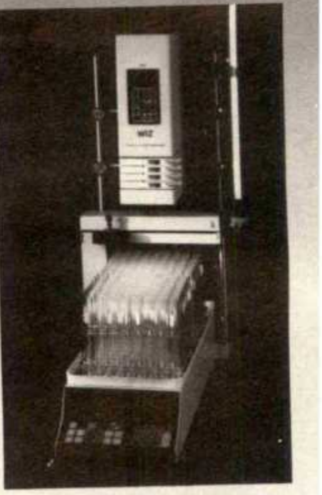

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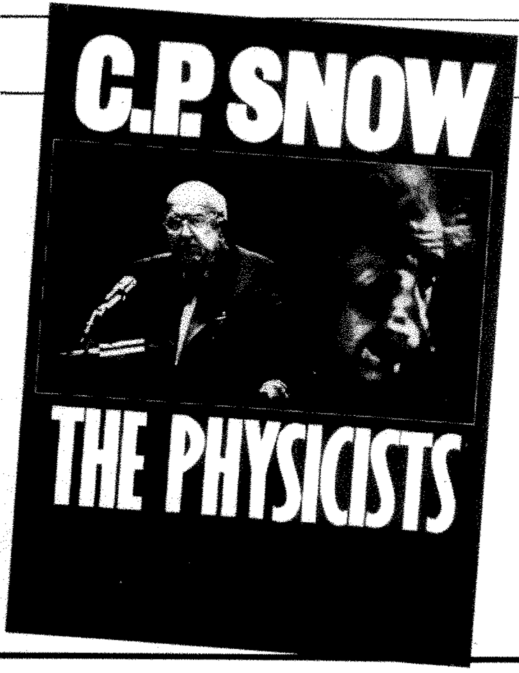

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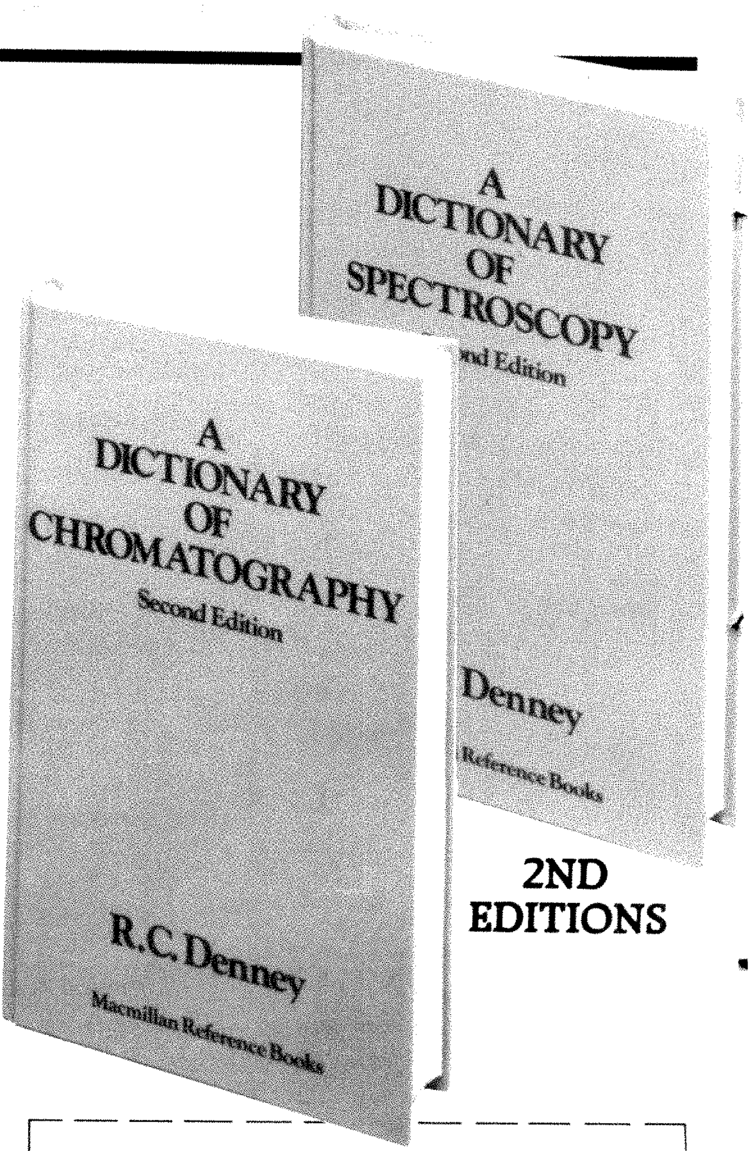
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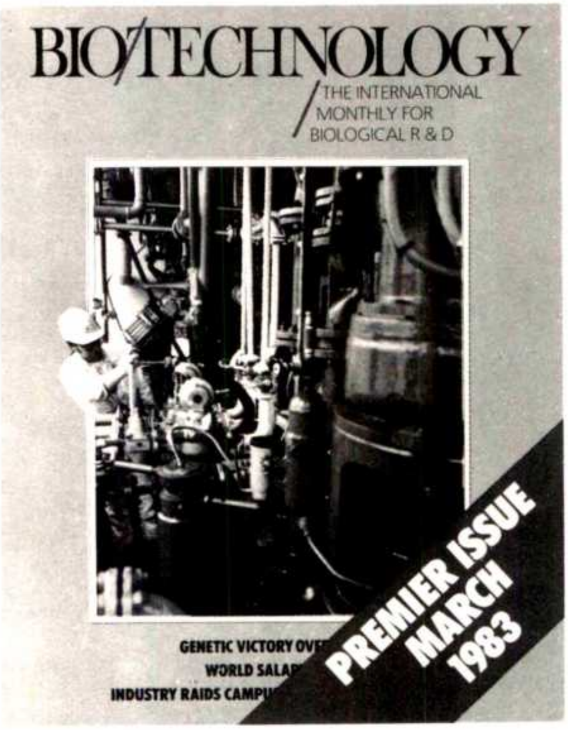

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Putting a case for civil science

The British government's Advisory Board for the Research Councils has made public its advice on spending money. In the process, it has unwittingly shown how its own work could be improved.

Even British governments, notoriously shy of giving reasons for their decisions, occasionally discover that openness does not jeopardize their survival or even offend against the laughable doctrine that only ministers of the crown take decisions. That is the most obvious lesson to be drawn from the publication last week (see page 3) of this year's advice of the Advisory Board for the Research Councils to the Secretary of the State for Education and Science, Sir Keith Joseph. Hitherto, this information has been as closely (perhaps more closely) guarded than that collected by the British intelligence services, on the grounds that it would be a great embarrassment if the responsible minister decided not to follow advice given. As it happens, Sir Keith Joseph seems to be the first minister to have disagreed with the board on how the budget for civil science should be divided. Now, to his credit, he has agreed that the advice from which he in part dissents should be open to public scrutiny. One result is a better appreciation of how these important issues are decided: too casually. Another is that the minister's hand will be strengthened in the battle to come with the British Treasury about the scale of research spending in the next three years.

The advisory board itself will benefit from the need to give persuasive arguments for its advice. Among the British government's battalion of advisory committees, the board is conspicuous because its most influential members have a direct interest in whatever advice is given. Since the board was first set up, after the Rothschild inquiry in 1971, its chief function has been to divide what the government is prepared to spend on civil science among the interested parties, principally the five research councils, each of whose chief executives is a member of the board.

The fear that if dog eats dog it may itself be bitten, frequently mistaken for gentlemanly politeness, explains why over the years the distribution of the science budget has changed only in minor ways. The Science and Engineering Research Council lost a little ground in the late 1970s, when high-energy accelerators and space research became unfashionable, but that balance is now in part to be redressed. The Medical Research Council was smart enough two years ago to recapture from the Department of Health the slice of its budget transferred to the government department in 1971. Otherwise, the advisory board has operated at the margins of its dependants' budgets, trimming a little here, adding a little there. Complacency in the face of adversity seems to have been the motto.

Publicity

Even the prospect of publicity seems to have helped to jerk the board out of this self-defeating groove. Its proposal that the British Museum (Natural History) should not now build a huge new exhibition space within the shell of its splendid Victorian building is not merely an economy (£23 million plus) but a public service. The recommendation that the budget of the Agricultural Research Council should level off in cash terms (and therefore shrink in real terms) after the next financial year will cause more serious damage. This council is more trapped than others by its commitments to its own staffs working in its own research institutes. Yet the board's case for letting agriculture carry the main burden of the economies necessary to finance future spending by the Science and Engineering Research Council would be more convincing if its own statement of priorities had been more persuasive. That the board should attach importance to "developments in biology" is natural enough, even if superficially inconsistent with its recommendation about agricultural research. To count as a priority "new developments in neuroscience" is sensible enough; but this goal is surprisingly not mentioned in the statements with which the research councils bid for funds. Did one of the board's members meet a neurobiologist on a train journey, perhaps?

Fashion

The board's first public account of its way of working also raises the suspicion that it is as liable as the next person to be carried uncritically away by fashion. Thus the board blesses with £22.5 million (over three years) the Science and Engineering Research Council's wish to become the basic research sponsor in a "national programme on information technology" about to be launched by the Department of Industry, and which may cost the council £50 million over the next five years. The snag, for readers of the board's account of itself, is that there is no way of telling whether this expensive exercise is supported by an assessment of ways in which money might usefully be spent on research or merely by the enthusiasm which Mr Kenneth Baker, a minister of state at the Department of Industry, has brought to his job as minister of information technology. Fashion of a different kind also accounts for the choice of "remote sensing" as another priority area of research: the chairman of the Natural Environment Research Council, Sir Hermann Bondi, is keen; meteorological observations from satellites are practically important, while oceanic observations will soon be an important source of data in oceanography; and civil servants can readily be persuaded that the objective is to find oil, or tin, or some other mineral.

Fashion seems also to have infected the board's report in its uncritical endorsement of (and collaboration with) the study of the relations between industry, universities and the research councils commissioned by the British government from the Advisory Committee on Applied Research and Development. That committee's terms of reference are hopelessly uncritical — essentially, to identify obstacles to making university research a handmaiden of British industry and to suggest how they might be removed. The committee appointed for this task appears to be aware that there are dangers as well as benefits in links between academic and industrial organizations, principally that universities may be made less able to dispense impartially their special kind of knowledge to all potential users of it. The Advisory Board for the Research Councils, custodian of basic research though it professes to be, says nothing on this point. But if the board is silent on the risks in too particular a relationship between universities and industry, who else can be expected to have influence?

These are the respects in which the board's first public report is deficient. The document also has merit. Last December, the Select Committee on Science and Technology of the House of Lords (see *Nature* **294**, 503; 1981) complained that the board had become too passive an instrument of policy, and the British government replied that the board should exert itself in throwing light on matters of public policy (see *Nature* 8 July, p.107). No doubt the flurry of activity there has been in the past few weeks in

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setting up inquiries into topics such as the relations between research councils and universities is one sign that the board has woken up. Another is an important discussion, now published, of the demographic problems presented by the maldistribution of university teachers in most disciplines, a consequence of the rapid expansion of the 1960s. As elsewhere, there are too few young people and too many who are middle-aged (see chart, p.8). Much of the redirections of science spending planned for the next few years is intended to redress this balance, and not before time. The obvious danger is that by using money intended for research to improve the condition of British universities, the research councils may be helping to break the board's rule that they should not attempt to make good the deficiencies of the dual-support system. The snag is that self-restraint in that regard requires not merely that the councils should back good people, but that they should do so only if those people are well placed to carry out research effectively. So why should not the board spell out the criteria it expects that its dependants will follow?

A more awkward question needs to be taken up (in next year's review?): are the board's dependants as efficient as they should be? In the past few years, the peer-review systems operated by most of the research councils have won widespread respect, but many of their own research establishments fall far short of what they might be. Perhaps the most conspicuous example is the Medical Research Council's National Institute for Medical Research, which has long been balkanized into "divisions" that hardly ever speak to each other and where even minor items of expenditure must be met by cheques paid from headquarters. The board now repeats the research council's promise that its institute will be "restructured", and has appointed Dr D. A. Rees to that unenviable task. But who is to say that this one institute is not just the visible tip of a much larger problem?

Old-fashioned ways of delegating financial responsibility are certainly not confined to one institute or even to one research council. And all those that depend to some extent on contracts with government departments for the conduct of applied research are necessarily caught up in a complicated set of negotiations that only occasionally leads to a change in the pattern of expenditure. Similarly, the recruitment of extra staff within the research council system is hampered by rules dictated from on high which are designed to limit the total strength of the public service, but which prevent research council establishments from earning honest money when they can. The British Prime Minister's favourite cost-cutter, Sir Derek Rayner, is thought recently to have turned his attention to the research councils. It will be best for everybody if he discovers that the present system is too much hamstrung from outside.

The most serious defect in the working of the advisory board, now revealed, is however the incompleteness of such authority as it enjoys even in the field with which it is concerned. (The case that there should be some mechanism for overseeing the whole of publicly-supported research, defence research included, is irresistible but nevertheless repeatedly denied.) The board's membership includes most of the chief scientists of government departments which are in their own right substantial sponsors of research, much of it in universities, little of it different in kind from work sponsored by the research councils. The British government has firmly rejected the argument of the House of Lords Select Committee that there should be some device for overseeing the whole of public sponsorship of research and development, partly on the grounds that the present prime minister has a personal interest in the subject. But can it make sense that government departments, from health to industry, should be caught up in a semi-judicial (but over-casual) assessment of what the research councils should be allowed to do when their own spending in similar fields is innocent of outside inquiry? The simple answer, which all bureaucrats will echo, is that what the advisory board calls the science budget is a charge on the Department of Education and Science, and that there would be departmental barriers to a more wide-ranging inquiry. But it makes no sense that such procedural matters should inhibit what good sense requires.

Vatican and the bomb

A group of scientists has given the Pope a tactless declaration on nuclear warfare.

The Pontifical Academy of Sciences is not an academy in the strict sense (its members are invited by the Vatican) but it seems well on the way to being pontifical. The statement put out at the end of September is thus a sombre warning of the dangers of nuclear war from which few will dissent, a stern proscription of steps that might trigger off such a war and an appeal to those concerned — politicians, scientists, religious leaders "and other custodians of moral principles" — to use their best influence for the avoidance of nuclear war. The statement is a remarkable document if only because of the distinction of those who have signed it — the list includes, for example, the presidents of the National Academy of Sciences of the United States (Dr Frank Press) and of the Royal Society (Sir Andrew Huxley), acting in their personal capacities. Its weakness is that the pursuit of agreement on a set of statements about nuclear war with which nobody can quarrel seems to have obscured the more immediate issues that must be dealt with.

Even the analysis of the problem of nuclear warfare in the published statement falls short in clarity of what the members of the group individually would suggest. Thus the statement says that "there appears to be a growing fatalistic acceptance that war is inevitable and that wars will be fought with nuclear weapons". Can that be so? At a time when there are more (and more hopeful) negotiations on the limitation of nuclear weapons under way than in the past three decades? Or when military authorities are seriously considering the possibility of defending Western Europe with conventional rather than nuclear weapons? It is true, of course, that political relations between East and West in Europe have deteriorated in the past year, for which reason it is all the more remarkable that the arms negotiators in Geneva are still talking to each other, however desultorily. Similarly, it does not follow, as the Pontifical Academy of Sciences suggests, that "proliferation of nuclear weapons to additional countries seriously increases the risk of nuclear war and could lead to nuclear terrorism". If anything, the argument should be turned around: as deep and apparently irreconcilable conflicts between sovereign powers become embittered (the Middle East?), the danger that one or other party will seek nuclear weapons increases. Proliferation (of which there has been mercifully little so far) is more likely to be a symptom than a cause.

The academy's recipe for action is similarly clouded. September's statement sanely asks that nations should renew their efforts to negotiate effective arms control agreements. The distinction of the participants should carry some weight, both in Geneva and in the continuing anxiety about the design of more effective measures for safeguarding the civil trade in nuclear power. But national governments are also asked "never to be the first to use nuclear weapons". Can that be seriously intended? For most of the signatories of the document must be fully aware that this is an extremely contentious issue. In Europe, the Soviet Union has repeatedly offered a "declaration of no first use", and its Western opponents have as steadfastly refused a deal. The reasons, moreover, are well understood. Either such a declaration means nothing, or it will favour that adversary in some future conflict which is weaker in conventional forces. To couple an appeal for more serious negotiations on arms control with a declaration calculated to lose the sympathy of many of those who will read the statement is tactless, to say the least.

What can be accomplished by such manifestos? There are two choices. Either a statement on some public issue can be so penetrating and original in its analysis of a problem, or so arresting in its description of it, that ordinary people will stop and think. Or it may so powerfully suggest new things to do that statesmen will be compelled by sheer logic to follow the course prescribed. This statement on nuclear warfare is, unhappily, neither one thing nor the other.

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British research cake cut painfully

First public review reveals dissenting voice

Dissent among the five research councils has for the first time marred the customary gentility of the annual recommendations by the Advisory Board for the Research Councils (ABRC) on the British science budget. In what is meant as fighting talk, the Agricultural Research Council (ARC) says it "cannot associate" itself with the proposal that its own budget should take a declining share of the funds available beginning in the financial year 1984–85.

Ironically, this row has surfaced on the first occasion when the board's advice to the Secretary of State for Education and Science (Sir Keith Joseph) has been published. Sir Alec Merrison, the board's chairman, says that the row is unprecedented. The recommendations now made are provisional in that they will have to run the gauntlet of the British government's annual expenditure review, now under way. Sir Keith Joseph, the minister, has, however, already lopped £6 million from the proposed budgets of the Social Science Research Council (SSRC), asking that this sum should instead be used for recruiting young scientists to university posts, and has quickly fallen in with the recommendation that the British Museum (Natural History) should not spend in excess of £23 million to extend its exhibition space at South Kensington.

The recommended budgets for ARC imply a constant contribution from the science budget of £46 million in each year for the three years beginning next April, implying that the council will have to make good the unknown consequences of inflation by internal economies.

This parsimony towards ARC (and to a lesser extent towards the Natural Environment Research Council (NERC)) stems from the board's view that funds within the science budget should be diverted to other better causes — an increase in real terms of the funds available to the Science and Engineering Research Council (SERC) to support initiatives in fields such as information technology and for the provision of posts for younger scientists now frozen out of the research system.

Merrison explained last week that ARC had been singled out because of the large proportion (just over a half) of its budget channelled through the agriculture ministry to support commissioned applied research and because its links with universities are weaker than those of the other research councils.

But ARC has not yet lost hope. At a recent meeting the council endorsed the formal dissent of its secretary, Dr Ralph Riley, from the ABRC recommendations, and in the next few weeks will be asking Sir Keith Joseph to take a broader view of national priorities in general and ARC's research enterprise in particular. If more funds are not found, ARC reckons that it will have to lose 300 staff over the next three years, of whom 200 will be scientists, and that in 1984–85 it will be spending £7 million out of a total budget of £107 million to meet the cost of staff pensions and severance pay.

ABRC's argument is guided by its explicit choice of the fields in which publicly supported civil science should be encouraged: biology (especially biotechnology), remote sensing, information technology, marine resources and the neurosciences. But the board also says that its cutting of the cake this year reflects the view that the retreat from big science (experimental high-energy physics, space research) begun in the 1960s has gone far enough, and its general concern for the well-being of research and researchers in universities.

Running through the board's statement is its theme-song for 1982 — that funds spent on civil science, in universities or elsewhere, should be directed with industrial needs in mind. Its report says that it has "readily agreed" to help carry out a study (jointly with the Advisory Council on Applied Research and Development) whose terms of reference apparently leave no room for consideration of the snags that such collaboration may encounter. ●

Harvard accused of impropriety

Washington

A government audit has concluded that Harvard Medical School improperly charged $1.7 million to federal research grants from 1975 to 1977 and failed to document a further $29 million charged during the same period.

The findings by the Department of Health and Human Services (HHS), which runs the National Institutes of Health and the Public Health Service, were the latest salvos in the battle that has been waged between Harvard and the government during the five-year federal investigation of Harvard's management of grants. Last year, an HHS audit of Harvard's School of Public Health found $2.1 million in improper charges by the university.

Asked whether Harvard would repay the sums in question, the university's director of auditing, Anthony Woodworth, said, "I very much doubt it". He said both audits are the subject of negotiations with HHS officials, and that they merely represent "recommendations" to the government, not orders for repayment.

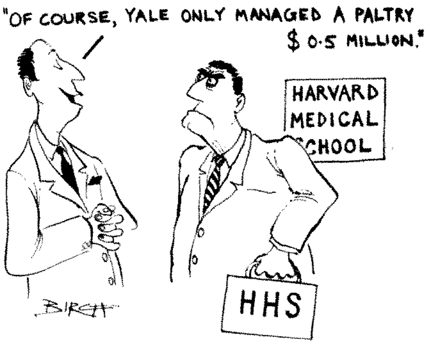
Woodworth said that an independent audit by the accounting firm of Coopers and Lybrand of Harvard's management of grants in fiscal year 1978 "gave us a clean bill of health". He suggested that HHS's release of their recent findings was a public relations stunt.

The HHS audit specifically claims that the medical school improperly transferred $1.6 million in charges from one project to another to cover cost overruns and to ensure that all available grant money was spent. The auditors said that some $3 million was charged without adequate documentation, and that the validity of $26 million that went to pay salaries could not be verified because of poor records.

Harvard appears to be trying to suggest that the dispute is one of academic freedom versus government meddling. Harvard and other universities have invoked academic freedom in resisting government efforts to require better accounting of how a faculty member divides his time between teaching and various research projects.

Harvard's attitude clearly annoys HHS. As one government official said, "What I find amazing is that we become the 'bad guys' for trying to reclaim the taxpayers' money".

Dr Delphis Goldberg, of the House Government Operations Committee panel that oversees HHS, said that the audits found problems that went deeper than the dispute over effort reporting by faculty. At the School of Public Health, he said, "there has been a substantial diversion of moneys" from specific grants to totally unrelated research projects, even projects under different principal investigators.


"OF COURSE, YALE ONLY MANAGED A PALTRY $ 0·5 MILLION."
HARVARD MEDICAL SCHOOL
HHS
BIRCH

The investigation of Harvard grew largely out of charges by Dr Phin Cohen, formerly an assistant professor of nutrition at the School of Public Health. In 1976, he accused the school of diverting funds from his NIH grant to other unrelated projects. A subsequent NIH audit confirmed his accusations — Harvard was required to return $132,000 — and recommended a full-scale audit of Harvard research grants by HHS.

Stephen Budiansky

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NSF budget for 1983

Spending lags behind inflation

Washington

With so much of the federal budget still in doubt for the fiscal year that started in October, the National Science Foundation (NSF) considers itself fortunate that both houses of Congress have approved an appropriation bill, despite their differences, and sent a recommendation to the President which he signed before the end of of the old fiscal year.

Unfortunately, authorizing legislation — which spells out how the money should be spent — had been passed only by the House of Representatives, while because of a disagreement over who should look after NSF, two different Senate committees have worked up two different versions of NSF's authorization for fiscal year 1983. If the "lame-duck" Congress that returns later this month does not repair this situation, NSF may have to operate during the remainder of the fiscal year without detailed guidance — as it did last year.

The final amount appropriated was $1,092 million, a little more than the President's request of $1,078 million, but still close to the levels of the previous two years of $1,003 and $1,036 million respectively. With inflation, this means a net decrease.

The bulk of the money, $1,060 million, will go to NSF's research and related activities. This includes $14 million in funds taken out of other programmes which NSF can spend at its discretion.

Pre-college science education programmes, which the Administration tried to "zero" (a common verb in budget director David Stockman's vocabulary) survived with $15 million. The total NSF science education budget will be $30 million, the other $15 million going to education fellowships requested by the Administration. The House of Representatives Appropriations Committee, more sensitive to the national "crisis" in science teaching, wanted $25 million spent on pre-college education, and the Senate put in none: so they compromised.

The behavioural and social sciences were less successful in their attempts to resist Administration cutbacks. In 1982 the Administration tried to cut funds for these fields, but the social scientists fought back in Congress, and some of the money was restored. This year, the Administration requested $36.6 million — and presumably that is what will be spent by NSF — but the funding is still below the 1980 level of $47.9 million.

Among other decisions, both appropriations committees required that NSF should obtain their approval before spending money on the new ocean drilling vessel, *Glomar Explorer*. The conversion of the Central Intelligence Agency's vessel to a research ship has been very controversial and the committees wanted to see the results of the latest engineering studies. The $14 million requested to operate the existing *Glomar Challenger* was reduced to $12 million.

In September, just as researchers planning to go to Antarctica for the summer season were getting ready, the Senate Appropriations Committee cut the US Antarctic programme's $86.4 million budget request by $15.3 million. This reduction would have cut the season's scientific programme by 60 to 70 per cent. In the end, only $4 million was taken from the programme, leaving $73.4 million for running costs and $9 million for science. The cut will delay some construction planned for the base at McMurdo and mean later relief for the wintring-over parties in August 1983.

International programmes at NSF continue their recent decline, from $13.7 million in fiscal year 1980 to $9.1 million in fiscal year 1983. The foundation has circumvented the Administration's and Congress's dislike of international science by funding international scientific travel out of the regular research funds. But because it puts international travel in competition with so many other things, it may accelerate the well-documented trend of declining US scientific travel abroad and fewer promising young US scientists taking fellowships overseas. **Deborah Shapley**

Slaughter steps down

John B. Slaughter, director of the National Science Foundation, stepped down on 1 November in accordance with his decision several months ago to become chancellor of the University of Maryland College Park Campus. Since the Administration has not yet decided on a successor, Donald N. Langenberg, NSF's deputy director, is to become acting director of the foundation. Langenberg is likely to be a leading candidate for the position of director because of his extensive experience in various posts at the foundation.

Deborah Shapley

Research on ageing

US physicians come to life

Washington

Research on ageing has received some welcome attention in past weeks. First there was a meeting about recent research findings held at Rockefeller University, New York and sponsored by FIBER, the Fund for Investigative Biomedical Research, a relatively new private organization sponsored by medical philanthropist Mary Lasker and others. Then, in late October, the Institute of Medicine (IOM) devoted a day of its annual meeting to ageing research and the challenge to the US medical systems of the growing proportion of old people in the population (see *Nature* 26 August, p.779, and 2 September, p.2).

Both meetings were rich in suggestions from researchers in other fields for possible lines of inquiry into the mechanisms of ageing and into ways of prolonging cellular life or at least retarding decay. For example, at the FIBER meeting, Dr Allen R. Goldstein, chairman of the biochemistry department at the George Washington University School of Medical and Health Sciences, described the progress that has been made recently in understanding of the thymus: some recent work suggests that thymosin, which has been used to restore the immune response in cancer patients treated with chemotherapy, could also boost the immune response in older individuals. Joan Smith-Sonneborn of the University of Wyoming, a zoologist, described how paramecia can repair their own DNA through photoreactivation, and thus live a little longer.

Professor James Williamson, of the department of geriatrics at the University of Edinburgh, talked about the social and financial problems posed by the large numbers of elderly people that will survive later in the century. Professor Williamson runs a geriatric service which consists of an interdisciplinary team of doctors and nurses. Patients referred to the service are visited at home within a day; only 30 per cent of them have to go to hospital and then usually for only a short time, or for regular short visits to give relief to their families. Such a service can reduce the number of old people in hospital geriatric wards by as much as 50 per cent.

A doctor from Los Angeles said that such a system would be impossible there, but Paul Beeson, editor of the American Geriatric Society's journal and formerly chairman of the department of medicine at Yale University medical school, disagreed. He said that most US doctors had their ideas of medicine shaped by the 1950s and 1960s, when funds were growing for biomedical research and specialities that could bring spectacular results were most sought after. Several leaders of US medicine have attacked the notion of geriatric medicine as not being medicine at all, he said, because it aims only to help people — and their families — and is more a social service than an investigative science.

Recommendations about how US medicine should address the problem of the nation's ageing population may emerge from a study that the Institute of Medicine and the National Academy of Sciences have set up and which is due to be completed next year. **Deborah Shapley**

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Madrid conference

Failing hopes for agreement

The review Conference on Security and Cooperation in Europe (CSCE), which reconvenes in Madrid next week, seems fated to be as inconclusive as the previous gathering in Belgrade (October 1977–March 1978).

The implementation of the Helsinki Accords has, if anything been hampered by increasing international tension, and, in particular, the imposition of martial law in Poland. So far there has been no agreement on the further development of the CSCE process. A possible draft statement (Document RM-39) drawn up in December 1981 by the non-aligned and neutral signatories of the Helsinki Final Act remains in abeyance, because the delegations from the United States and its Western allies have so far refused to discuss it while the situation in Poland shows no marked improvement.

The linking of negotiations on security and economic and scientific cooperation with those on human rights is one of the most sensitive issues in the Helsinki process. During the drafting of the Helsinki Final Act, the various proposals for cooperation were divided into three large filing baskets — security and arms control (Basket I), economic, scientific and technological cooperation (Basket II) and human rights (Basket III).The contention of the United States and some of its Western allies is that the signatory states must implement all three baskets. The Soviet Union and socialist bloc, however, prefer to treat the three baskets separately.

After the Belgrade review conference, it was decided to hive off scientific and technological cooperation — then considered the least contentious subjects — into a special "scientific forum" in Hamburg, held a few months before the main conference opened in Madrid.

The contents of Document RM-39 are for the most part acceptable in principle to all sides, although some of the "confidence building measures" associated with Basket I would doubtless need further detailed negotiation. (Thus the French proposal for an extension of the zone in which the Soviet Union should notify major troop movements back to the Urals has been countered by a Soviet demand that the NATO powers should notify major naval movements in the Atlantic.)

The Helsinki process, however, as the United States in quick to point out, is not a matter of legal wrangling but the creation of an atmosphere of detente. In the absence of such an atmosphere, they stated last March, it is impossible to discuss RM-39 or any other draft agreement. Since then the international climate has deteriorated even further. United States sanctions against the trans-Europe pipeline has evoked Soviet criticism that the United States is deliberately trying to sabotage detente and urging that the pipeline is a success for Basket II.

The disappearance of the Helsinki Monitoring Movement in the Soviet Union (the last group, reduced by imprisonments to two members, dissolved itself last summer) is unlikely to improve the situation in Madrid. Nor is the recent renewed attack in the Soviet media on Anatolii Shcharanskii, a former member of the movement, now serving a 13-year sentence for human rights activities which the courts found treasonable. Nor is the arrest, in Poland, of the mathematician Dr Zbigniew Romaszewski, who in 1980 compiled and sent to Madrid a report on human rights abuses in Poland.

Under these conditions, it is uncertain whether any useful discussions can be expected from the forthcoming session. If the United States once again refuses to discuss Document RM-39, there would be little point in continuing the session. Sooner or later, if no final statement is agreed, the Madrid Conference will go the way of its predecessor in Belgrade; it will have to agree to disagree, and set a place and date for a new Conference. **Vera Rich**

UK university mergers

No links for UMIST and Salford

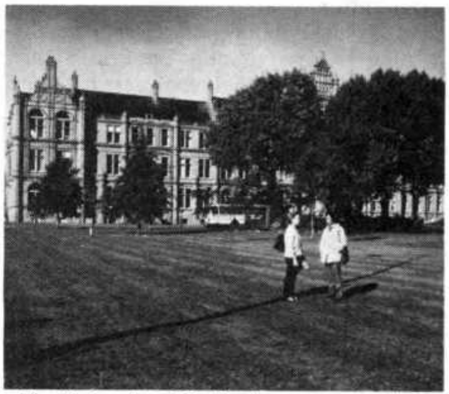
A combination of institutional loyalty and academic snobbery seems to have frustrated one of the most promising of amalgamations between British universities — a much canvassed proposal that the University of Salford and the University of Manchester Institute of Science and Technology (UMIST) should join forces. Two weeks ago, the UMIST governing body (its council) decided that, among several possible options, it would prefer to forge stronger links with its parent university, the University of Manchester.

Both partners in this abortive marriage have just lived through a hard year. Salford has been adjusting to the prospect of a sharply reduced subvention from the University Grants Committee (UGC), discovering that the extension of the transitional period has not allowed it to avoid painful retrenchment. UMIST, three miles away, has spent the past year wrestling with the problem of whether its principal, Professor R.N. Haszeldine, should remain in the post to which he was appointed in 1975. The cost of modernizing the principal's residence was a contentious issue. Professor Haszeldine, distinguished for his research on fluorine chemistry, has retired early with effect from the end of this year.

Almost immediately that issue had been resolved, UMIST found the local newspapers full of a proposal by Mr Fred Sylvester, one of Manchester's Members of Parliament, that Salford and UMIST should agree to merge. The logic of such an arrangement seems irrefutable; both institutions concentrate on science and technology (but have important outside interests, in politics and modern languages respectively, for example) while they are physically separated by only three miles.

The University of Salford now says it is disappointed that the prospect of a merger between the two institutions has disappeared. People at UMIST, on the other hand, profess themselves offended that they first heard of the proposed merger from newspaper reports, and that there has never been a formal approach from Salford.

The preferred option of the UMIST court to strengthen links with the University of Manchester, is intended to build on the institute's constitution as the Faculty of Technology of the university. The university awards degrees to graduates of the institute, but otherwise relations between the two over the past thirty years have been close or distant according to the personalities of those in charge. UMIST now cites as an example of the collaboration foreseen with its parent institution the occupation of the same building by two departments of metallurgy, its own and that of the university, saying that it has asked for further discussion of such cooperation.



The University of Salford — to remain alone

By common consent, Salford has survived the hardships of the past two years with flair, largely because of the success as vice-chancellor of Dr J. M. Ashworth, the most centrally placed (but not the most senior) of the British government's scientific advisers until the end of 1980. One Salford academic said this week that Ashworth has given Salford such a sense that it has a future that hope is now pinned on the belief that UGC will next year relent.

Both institutions are on course in their reduction of student numbers, while each of them has increased the intake of overseas students in the current academic year, Salford by 46 per cent and UMIST by half as much. ●

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US medical schools

Counting social costs

Washington

There is a story making the rounds of college administrators in which a university president is greeted by the Devil at the gates of Hell with an offer of the presidency of the local university. Delighted, the president immediately accepts, remarking that he had expected far worse treatment. "Yes", Satan replies with a glint in his eye, "but our university has *two* medical schools."

If that is indeed the American university president's personal vision of hell, then some recent changes in government health-care financing may be a foretaste. Stanford University president Donald Kennedy, for one, has left no doubt that the federal government is taking the part of the Devil in imposing limitations on the reimbursement to hospitals for treating poor and elderly patients covered by government programmes.

Kennedy's views are shared widely among university-affiliated teaching hospitals, which see themselves as innocent victims of government's efforts to control rocketing health-care costs, and in particular those costs borne by the government itself.

In the past ten years, federal spending on Medicare (which covers the elderly through the social security system) climbed from $8,800 million to more than $44,000 million; spending for Medicaid (which covers the poor through the welfare system) rose from $4,700 million to more than $17,000 million. Even before the coming of Reagonomics, these seemingly uncontrollable expenditures were a matter of concern within Congress and the executive branch.

But to the universities, even these modest cuts have been a source of worry. According to a recent report prepared by the Association of Academic Health Centers, Medicare and Medicaid payments to university-affiliated teaching hospitals allowed them to expand their faculties and their educational programmes during the late 1960s and 1970s. This dependence has made them acutely sensitive to any efforts to limit reimbursements.

So, with some new and much tougher limitations in effect from 1 October, the complaints from universities have grown louder. Kennedy, in his Harvard speech, called the government policy "disastrous". The crux of the matter, he said, is that the government is no longer reimbursing hospitals for the full cost of Medicare and Medicaid patients. This forces the hospitals to raise their charges to private patients, and puts a disproportionate burden on teaching hospitals, which receive a high proportion of difficult and charity cases. Furthermore, the higher costs to private patients at teaching hospitals means that more of the routine — and profitable — business is going to county and private hospitals.

The shifting of costs from Medicare and Medicaid patients to private patients has been well-documented by the insurance industry, which ends up paying more. According to Neil Swan of the Health Insurance Association of America, the government shortfall will amount to $5,800 million this year. Kennedy said that charges to private patients at Stanford University Hospital could be reduced overnight by 14 per cent if Medicare and Medicaid reimbursements covered true costs.

And data from the Association of American Medical Colleges (AAMC) seem to support the universities' contention that they carry a greater burden than most. A comparison of the 327 member hospitals in AAMC with the 5,503 non-AAMC hospitals in the American Hospital Association found that although the teaching hospitals accounted for 26 per cent of total net patient revenue, they carried 35 per cent of bad debts and 47 per cent of the charity write-offs.

The new rules impose, for the first time, a limit on the annual increase in all hospital costs paid for by Medicare and Medicaid — a limit that will widen the gap between government reimbursements and the true costs of treating government-supported patients. The increase for 1983 has been set at 7.9 per cent; hospitals whose costs rise more than that will have to bear part of the excess themselves. The new rules also require that among groupings of similar hospitals, no-one can run costs greater than 120 per cent of the group's average. (That limit will drop to 115 per cent in 1984 and 110 per cent in 1985.) Together, these measures are expected to save $480 million in 1983 and $3,770 million by 1985.

The universities' complaints fall on less-than-sympathetic ears at the Senate Finance Committee, which, at the instigation of Senator Robert Dole (Republican, Kansas), wrote the legislation ordering the new rules. One staff member said "you hear that griping all the time", but that the rules contain several special provisions that favour the universities. Most important is exemption from the new limitations for direct educational costs of teaching hospitals, for example, interns' and residents' salaries.

The new rules do contain, however, a direct blow to one bountiful source of funds for the universities under Medicare and Medicaid: faculty practice fees. These are fees paid for the professional time of faculty physicians who treat Medicare and Medicaid patients. The faculty physicians turn these reimbursements over to the university, which uses them partly to supplement faculty salaries and partly for "dean's funds" that pay educational expenses, such as equipment purchases. Under the new rules, faculty practice fees cannot exceed the faculty member's salary.

The situation is complicated by a proposal made public earlier this month by Richard Schweiker, Secretary of Health and Human Services, that would completely overhaul the method of government payment under Medicare and Medicaid. This plan, known as "prospective reimbursement", would set reimbursement rates in advance. The most serious reservation is that, like the 1 October rules, it does nothing about the problem of the cost-shift to private patients. The insurance industry, which is particularly eager for action, points to successful state programmes (in Maryland and New Jersey, and soon in Massachusetts and New York) that combine prospective reimbursement with state regulation of charges to all patients.

Stephen Budiansky

Lead in petrol

Europe too soft?

Brussels

A new attempt is being made to revive the debate about lead safety levels by European consumer and environmental groups. Despite numerous directives defining safety levels in everything from the workplace to shellfish, EEC is now being accused of setting lower safety levels than either the United States or Japan. Calls for a revision of European standards of lead in petrol were made last week by European associations of consumer groups (BEUC) and environmental groups (EEB).

Since 1978, an EEC directive has permitted lead in petrol levels of a maximum of 0.4 grammes per litre and a minimum of 0.15 grammes per litre. The United Kingdom's decision to enforce the lower level from 1985 onwards aroused considerable controversy earlier this year because of scientific evidence that even low concentrations of lead in air could have harmful effects on the nervous system of critical groups such as children and pregnant women.

A new campaign is being launched to persuade the European Commission to revise the directive, and to introduce lead-free petrol throughout the member states. No member state is at present allowed to do this and pressure is building up from inside and outside the European Community. Germany and Denmark are in favour and Sweden, Norway and Switzerland are threatening to take unilateral action. The consumer and environmental lobbyists argue that if the two other main car-producing countries are convinced of the desirability of lead-free petrol, so should be the Community. Japan started as early as 1968 and the United States began to phase out leaded petrol in 1974, although small refineries are still allowed to produce leaded petrol.

Experience in both Japan and the United

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States suggests that there are few penalties for introducing lead-free petrol. The added cost to the consumer in the first two or three years is probably no more than £0.8 pence a gallon, according to the UK Ministry of Transport, while the lobbyists claim that studies in the United States and Canada prove that there are definite savings in vehicle maintenance costs of perhaps $0.05 a US gallon. In addition, European car manufacturers already make cars for the US and Japanese markets designed to run on lead-free petrol.

But industry is unlikely to be happy about being asked to make new investments when there is already a massive overcapacity in European refineries and the car industry is faced with a prolonged recession.

Their defence rests mainly on placing doubt on the scientific evidence of low concentrations of lead in air causing damage. While EEB and BEUC admit there is unlikely ever to be exact correlations between, for example a reduction of lead air concentration and the number of mentally subnormal children in the EEC, there is enough evidence to warrant action.

The Isotopic Lead Experiment being sponsored by the Commission in Turin and Piedmont indicates that at least 30 per cent of the lead in blood comes from petrol, although there are, of course, plenty of other sources such as paint or plumbing. In the experiment, lead from Broken Hill Mine in Australia with an easily recognizable mix of isotopes 206 and 207 was put in the petrol sold in the area between 1977 and 1979 thus enabling the source of the lead in blood to be immediately identified. The full report of the research has yet to be released on the grounds that there has been some difficulty in interpreting the data, but there are now rumours that the real reason is the alarming nature of the conclusions.

The arguments will undoubtedly continue, though, on what constitutes a safe level of exposure. This year EEC approved a directive for a maximum blood lead level of 60 microgrammes per decilitre in the workplace despite calls from the European Parliament and some member states for more stringent standards.

The European Commission wants to review the blood lead reference levels which are the criteria for assessing the need to take remedial action. The reference levels are a maximum of 20 microgrammes per decilitre of blood for 50 per cent of the population, 30 for 90 per cent and 35 for 98 per cent of the population.

The Lawther Report in the United Kingdom, published in 1980, asserted that there was no convincing evidence of deleterious effects below the 35 microgrammes per decilitre level. Yet an American study by Davidow *et al.* in New York in 1982 on the influence of lead on the production of haemoglobin suggests that the blood lead level threshold below which children's haemoglobin production is not affected is 15–18 microgrammes per decilitre.

Jasper Becker

Blue skies all around

The pace of industrial investment in British academic research seems to be quickening. After several years of niggardly treatment at the hands of the British taxpayer, British academics appear to be more willing than ever that their research should be supported by industrial money. This is the spirit in which British Petroleum's Venture Research Unit, formed by BP Limited two years ago to support what is called "blue skies research", decided to invest £500,000 in four novel academic projects, three in Britain and one in Austin, Texas.

The four contracts agreed upon last week (but yet to be negotiated with the intended recipients) illustrate what BP means by blue skies research. One, with the University of Aberdeen, is an exploratory project further to investigate the possibility that L-phase bacteria may be able to transfer genetic material directly into the cells of plants.

The venture unit is also offering research contracts to the University of Sheffield to investigate the application of control engineering methods to the study of photosynthesis and to the University of Dundee for an investigation of the apparently superior properties of amorphous silicon as a material for semiconductors. The proposed contract with the University of Texas, Austin, is for the development of the work of Dr Jess Kimble on optically bistable devices, possible components in future computers.

Some of the score or so of projects so far backed are relevant to the immediate needs of BP, which maintains its own much larger in-house research programme for that purpose. According to Dr D.W. Braben, the manager of the venture unit, the objective is to find people who are preoccupied with some unusual problem and who appear to have the ingenuity to solve it. Many of the contracts so far signed are concerned with the mathematics of complex processes, with computer applications and with the behaviour of microorganisms. Under the usual arrangement with an academic group, publication is allowed until commercial exploitation is foreseen.

The University of Sheffield is also the partner in another industrial venture announced this week — the founding of a company (Plant Science Ltd) to exploit several applications of plant science developed at the university's Institute of Biotechnology, founded with a grant from the Wolfson Foundation in 1978 and converted into an independent unit (with a further grant of £360,000) in March 1981.

The new company has been started with a capital contribution from TDC, described as "the high-technology arm" of Finance for Industry, a kind of merchant bank supported by various clearing banks. Among the objectives of Plant Science Ltd will be the development of plant varieties yielding substantial quantities of chemicals (such as steroids) and of processes for their extraction. The technical director of the new company is Dr Michael Fowler, from the university institute, who with Dr Anthony Jubb (recruited as managing director from ICI Ltd) will share part of the equity with TDC and the university. ●

UK research councils

Spend, spend, spending

Next to the cost of defence and of the police, the British science budget stands out in British public expenditure by having increased in the past three years even more quickly than the rate of inflation. Sir Alec Merrison, chairman of the Advisory Board for the Research Councils, said last week that "you cannot stay in that position without paying a price". Part of the price may be the publication last week of the board's advice on how to cut the cake (£507 million in 1983–84, 1.2 per cent more than needed to compensate for inflation).

The board has the following comments to make on the cases put to it by its pensioners, five research councils, the Royal Society and a museum.

The Agricultural Research Council (ARC) plans to maintain and even increase support for university research, especially in biotechnology, reproductive endocrinology, plant growth hormones and food process engineering. A new computer centre is planned. If there were more money (there is not) it would be spent on the genetic manipulation of animals, vaccine production and the micro-propagation of plants.

The board says that ARC should press ahead with its priority projects and should seek outside sources of financial support for some of this but recommends a constant cash contribution from the science budget in the next three years of £46 million. ARC's total income would increase from £95.9 million in the present financial year to £107 million in 1985–86 on the assumption that the agriculture ministry would pay more for commissioned research.

The Medical Research Council (MRC) plans a scheme to rescue biomedical research groups left stranded by university economics up to a total of 20 groups at a cost of £50,000 each in each of the next five years, and a modest increase of graduate student support. The council promises "major restructuring" at the National

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Institute for Medical Research and at its Radiobiology Unit, and clinical trials in such fields as cancer treatment, NMR imaging and multiple sclerosis.

The board recommends that MRC should "review" its participation in the European Molecular Biology Laboratory (Heidelberg), warns the council not to spend research funds in neglectful universities and recommends that the council should have a constant 22.5 per cent of the science budget, amounting to £113 million next year which will be increased by about £5 million from other sources.

The Natural Environment Research Council (NERC) plans to support more university research and graduate students, and to continue diverting resources towards remote sensing. Participation in the international ocean drilling programme beyond 1983 is uncertain, given the likely 50 per cent increase of cost.

The board recommends a share of the science budget rising from £58 million in 1983-84 to £63 million in 1985-86, including a constant £0.4 million for ocean drilling. At the end of the period, NERC will be £1.7 million worse off than now, but will earn £29 million from commissioned research.

The Science and Engineering Research Council (SERC) plans to increase support for biotechnology and civil engineering, to cut back in marine technology and polymer engineering, to reduce its payroll by 1 per cent in each of the next three years and to divert resources of two "major new programmes" — information technology and space research, perhaps at a total cost of £50 million over three years.

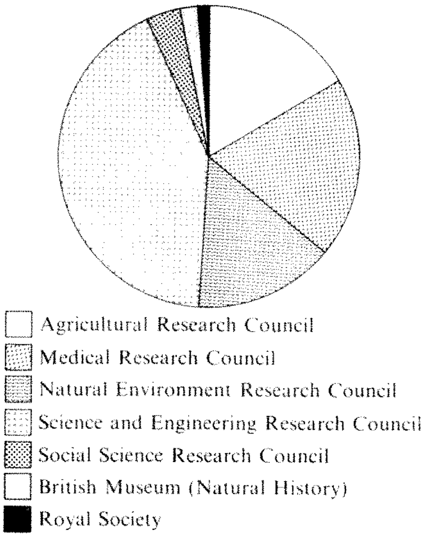
The board accepts that the time has come to halt the decline of "big science", recommends that the council should have an extra £22.5 million over three years to support basic research in information technology but that it should find the extra needed from its own resources. The board says that the council's budget should increase from £234 million now to £250 million next year and £278 million in three years, to which is added about £10 million in other income.

The Social Science Research Council (SSRC) pleads for a restoration of the funds cut from its budget in recent years, promising new research training schemes and boasting of a smoother organization. The board's recommendation accepts this case, suggesting a share of the science budget rising from £23.3 million next year to £25.5 million after three years. These are the sums from which the Secretary of State for Education and Science has subtracted in advance a total of £6 million. Sir Alec Merrison said last week that his board had no hard feelings on the subject, saying that the minister was the one with ultimate responsibility.

The British Museum (Natural History) asked the board for a general increase of funds to strengthen its general programme, and has been repaid with the recommendation that the proposed extension of the museum's exhibition space (at a cost of between £23 and £29 million) should be abandoned. The board says that the project, "however worthy, has no direct connection with scientific research".

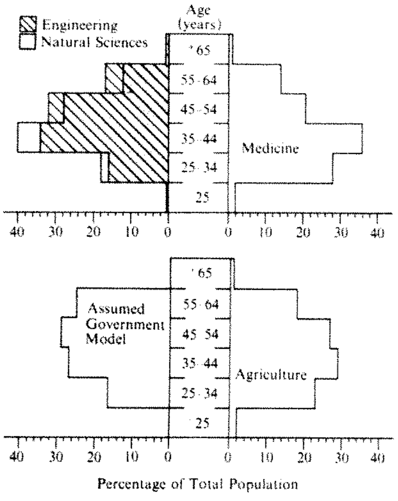
The Royal Society plans to spend £1 million a year on a scheme for 100 new research fellowships and asks for half that amount to support exchanges with the United States, while promising stronger collaboration with Japan. (The society laments that the Australian government is unwilling to help with the cost of the present Anglo-Australian exchange scheme.)

The board accepts the case that money should be spent on people rather than on instrumentation and project grants, and recommends an increase of £0.5 million in the society's budget to £5.0 million next year rising to £5.8 million in 1985-86. The society's total annual income is nearly £2 million greater. ●



Relative income of research councils, etc. in 1982-83. Figures include allocations from the science budget, commissioned research and other receipts. Total income is £579 million. (Source: *The Science Budget, a Forward Look*, the Advisory Board for the Research Councils.)

Age distribution in British academic disciplines.



Data from the ABRC report showing age distribution in various disciplines together with the steady-state distribution (bottom, left).

Environmental research at risk

Oceanography and forestry departments in British universities may be threatened by university efforts to trim spending in line with government cuts, according to Sir Hermann Bondi, chairman of the UK Natural Environment Research Council (NERC).

"It is of the utmost importance" writes Sir Hermann in the 1982 annual NERC report (HMSO, £4.25), published last week "that [the universities] manage their affairs in their present difficulties so as not to weaken their research capabilities . . .". Small fields, common to only a few universities, are particularly vulnerable, believes Sir Hermann — and he is clearly itching to tell the universities what to do without quite daring to. "Our ability to intervene and to help is clearly very limited" he writes. NERC has offered to help relocate departments from one university to another and to allow a degree of rationalization of resources in the universities — "but we must wait for their initiative . . .".

Sir Hermann has also had to wait for the initiative of certain government departments, not named in the report but certainly including the Department of the Environment, which have been very slow to define a long-term contract research policy. "Some of our customers find talking to us a little strange" said Sir Hermann last week. The strangeness is legal: advice has been given that a verbal agreement is as binding as a written one, with the result that "certain departments" have refused to discuss possible future funding or policy. This has led to considerable difficulties at NERC, which must assume that it always has the correct programme of basic research to provide expertise for any applied contracts that may be placed with it.

The Rothschild principle, where a portion of a research council's cash is obtained by contract from a customer department, was still "fundamentally right" said Sir Hermann. However, being right in principle was not sufficient — the department itself had to have great scientific competence. One of Rothschild's objectives had been to create that competence in the form of chief scientists' divisions — but in some departments the chief scientists had been pushed to one side. "They must be at the centre of decision-making" said Sir Hermann. The scientific attitude of the chief scientist should "permeate the whole department". **Robert Walgate**

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US geological research

Asset-stripping fear at survey

Washington

It has been a long, hot summer for the US Geological Survey (USGS) the United States' oldest basic research agency. A chain of decisions by the controversial US Secretary of the Interior, James Watt — that stem from a scandal in one part of the survey dating back to 1959 — had led by 1 October to the departure of approximately 2,400 people, including the entire Conservation Division and a third of USGS's marine geology research, to a new agency in charge of leasing federal lands, the Minerals Management Service (MMS).

One survey scientist describes his organization now as "a car going down the street and someone takes a wheel off". By October, Dallas Peck, the director of the survey who is one of its career scientists, had adroitly negotiated with Watt, who is his boss, to minimize the damage to the survey as a research institution, and cut back the "raid" of marine geology from Watt's original plans.

The loss of only a third of the marine geology group was considered a triumph of sorts by survey scientists who, over the summer, had been comparing departure plans. Morale was described as good, even though rumours were still circulating that Watt might now also ask the coal and marine scientists at the survey to come over to the politically-led MMS.

USGS conducts basic geological research, but since the nineteenth century it has also had some applied missions. The most important of these, in recent years, has been to make geological assessments of federal and Indian lands, both in the "lower 48" states and in Alaska, and of the enormous US continental shelf. These assessments are used by another agency of the Interior Department, the Bureau of Land Management, to second-guess oil and minerals company bids to explore and develop these lands, and for devising overall policies on which lands should be developed. USGS had the job of determining the "fair market value" of the lands and of specific tracts, to make sure the government made the corporations offer high enough royalties to flow to the federal and state coffers.

Both these tasks have been intensely controversial, and pitted the Interior Department, the Bureau of Land Management, the government, the companies, the environmentalists and the Indian tribes against each other in a fashion that makes the development of the North Sea and northern Canada look orderly by comparison.

The misnamed Conservation Division of USGS had the job of regulation, including royalty collection. Geologists in the Geologic Division, which includes the marine geologists, dealt with resource assessment and determining fair market value.

Over the years, many lands were leased for oil and mineral development in the Western states, and after the discovery of oil at Prudhoe Bay, Alaska, and the 1973 oil embargo, the leasing of lands grew, as did royalties. The Conservation Division did not have the manpower to handle these tasks. When Watt took office in early 1981, he unearthed a series of old General Accounting Office reports dating back to 1959, claiming USGS had failed to collect enough money.

Watt appointed a commission, headed by a businessman, David F. Linowes, to look into the loss of revenues. In January 1982, acting on the Linowes Commission report, he decided to transfer the handling of royalties to a new agency, the Minerals Management Service. But he went further than the Linowes commission and moved the entire Conservation Division — approximately 2,200 people — to make the bulk of the new MMS. MMS would handle the assessment, leasing and royalties collection for offshore lands, as well as the assessment, monitoring and royalties collection of onshore lands. Its powers over the onshore lands may yet be increased.

The transfer of the Conservation Division in January made big cuts in USGS, which had 10,000 full-time positions, and a budget proposed at $516 million. But it was considered by many to be an appropriate streamlining of the offshore leasing process, which had hitherto required many different agencies (or as one source says "35 people in the room with Watt") to coordinate in order to issue a single lease.

Far more controversial was another directive — a surprise to USGS and, apparently, to its director, Peck — that Watt sent in May asking that the Marine Geology Division, which makes offshore resource assessments, should also move over to MMS. Although the marine geology work consists of only 442 full-time positions and funding of $22 million, the division considers itself part of the scientific heart of the survey. Everyone was upset at the thought of these people, who pride themselves on being as good as university professors, working for the political MMS, which is run by Harold E. Doley Jr, a 35-year-old black Louisiana stockbroker who had helped get southern black votes for Reagan in 1980.

"All of a sudden, MMS would acquire all those ships and photographic equipment and scientists who were more senior than most of the personnel of MMS" comments one survey scientist — indicating the shock caused by Watt's memorandum.

Peck, however (who could not be reached for interview), took the blow in his stride, and argued that the agency was stronger. To his staff, in May, he said that "the focus of our activities is now even more clearly upon the scientific and technical aspects of our mission . . . It is now our responsibility to assure that we continue to earn the confidence which the secretary has placed in us. This will require that we look carefully at everything we are doing to ensure that our actions are responsible to the department's goals."

Peck negotiated with his bosses at the Interior Department all summer, and by the summer's end had reached an agreement by which only those marine geologists who really were involved in resource assessment and applied jobs would leave the survey for MMS. Finally, only 147 positions and $8 million left the programme. According to an earlier plan, marine geology was merged with energy, within the Geologic Division.

Harold Doley Jr — MMS director

Watt explained to a group of visitors to the White House a few weeks ago his belief that the government must facilitate the orderly development of the country's mineral and energy resources to avoid a "panic" rush to develop them in the twenty-first century.

The controversy, and continuing fear of another Watt raid on other researchers, has been high drama for the Geological Survey, which enjoyed a carefully nurtured respectable obscurity around Washington. But there are more important issues at stake, namely whether assessment should be carried out within a scientific agency if it is to preserve its integrity.

One commonly expressed fear is that MMS will be so anxious to respond to Watt's wishes that it will undervalue the tracts and not require high enough bids from industry — thus depriving federal and state governments of revenue. It will be some time before it can be determined if this major shift in science policy is a mistake, and loses the taxpayer money. If it does, it will be an ironic outcome for a process that began by trying to recoup funds for the public purse.

Deborah Shapley

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CORRESPONDENCE

EMBL's databank

SIR — We have had several comments concerning the recent letter from A. Slocum (*Nature* 7 October, p.482) describing a commercial timesharing service offered by Intelligenetics, Inc.

Intelligenetics makes available extensive software and computing capacity together with two nucleic acid sequence databases, including the one maintained by our group at the European Molecular Biology Laboratory (EMBL).

We wish to point out that the EMBL sequence data library has been sent to more than one thousand organizations, both academic and commercial. The data collection is supplied to everyone on the same basis: no restriction is made on its use or redistribution. Intelligenetics is free to offer access to the data in the context of their computing system, as is anyone else who may wish to do so. No-one has special or exclusive licence to the data: they remain freely available to any individual or organization directly from us.

Greg Hamm
Kurt Stueber
Graham Cameron

EMBL Nucleotide Sequence Data Library,
Heidelberg, FRG

Keep the index

SIR — Having read King's critical comments (*Nature* 30 September, p.390), I would like to express my approval of your biotechnology stock index.

The success of these corporations will reflect the ability of biotechnology to find a place in public and private business. There are many more biotechnology corporations, some without public stock offerings, that are just as important. It is not necessary to list every firm to achieve a general overview, as *Nature* has done so well.

I hope *Nature* will not hesitate to add or drop corporations from the index in keeping with trends in this field.

Squizzle Plekavich

Arlington, Massachusetts, USA

Preventing wars

SIR — I was pleased to read that J.R. Skoyles (*Nature* 7 October, p.482) approves of the proposals for a "comprehensive ban on all methods and means of warfare specifically designed to kill or injure by inducing human disease".

I feel that the criticisms with which he continues his letter are based on a misunderstanding of the second part of my letter (*Nature* 9 September, p.102). It was concerned only with the disarmament side of the disarmament/international security relationship[1], as is the UN draft Comprehensive Programme of Disarmament (CPD). The draft was referred by the UN Second Special Session on Disarmament back to the Geneva Committee on Disarmament for them to bring a revised version to the 1983 regular Session of the General Assembly. We believe that the content of the draft should be a matter for informed public discussion and comment, and wish to contribute to encouraging this.

The approach in the existing draft CPD is across-the-board disarmament in three (or four) stages. The arguments against this approach are: a 50-year history of political failure; the unrealistic requirement diplomatically and technically for simultaneous negotiations and verifications in all five categories of disarmament measures of Stage I; requiring the final stage of nuclear disarmament to coincide with the final stage of conventional disarmament, despite uncertainty on whether world society can achieve the latter; and the preclusion of a selective comprehensive treaty banning non-nuclear weapons of mass destruction and all disease-inducing methods of warfare, which is the only immediately feasible treaty of comprehensive disarmament[2].

The specific measures mentioned in the second part of my letter were only suggestions of what hopefully might follow the conclusion of such a treaty, if the disarmament diplomats could be persuaded to replace their "disarmament in layers" by a more meaningful approach.

Jeffrey Segall

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1. UN. A/36/597, 19 November 1981.
2. Meeting of Professions for World Disarmament and Development, 2 Oct. 1982.

The fetal soul

SIR — I beg leave to disagree with the comment in your leading article that "gastrulation is the latest point at which twinning can occur and thus the first point at which one body can have a unique soul" (*Nature* 7 October, p.475).

In one of the most solemn declarations of the Catholic Church Pope Pius IX in 1854 declared:

> The blessed Virgin Mary was, in the first instant of her conception, through the merits of Jesus Christ the saviour of the human race, by a singular grace and privilege of God almighty, preserved immune from every stain of original sin.

It is thus certain for Catholics that the soul enters the fetus at conception. If the physical fetus divides, God is as capable of creating a second soul as He was of creating the first — thus both bodies will have immortal souls, each of which will eventually spend eternity in a state of friendship with God (Heaven) or enmity with God (Hell).

Dave Parry

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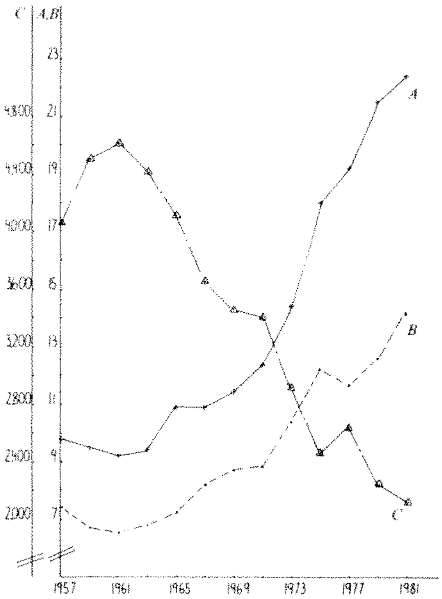
Costly journals

SIR — Robert Campbell was right to point out in an introduction to New Journals Review (*Nature* 7 October, p.49) that falling circulations do bring sharp increases in journal prices, thus causing a further drop in subscription levels, and further price increases. It is a problem we have been interested in for some time now[1] and have studied quantitatively in relation to physics periodicals. The problem facing libraries is indeed difficult. Taking our sample of 160 journals as an example, if a library wanted to keep up with both the growth in the number of core physics journals and their rising prices, it would have needed to increase its subscription budget by a factor of 30 since 1957.

We have shown elsewhere[3] that most of the price increases can be attributed to falls in circulations, but we have also demonstrated that, for the last few years, journal prices have been rising in anticipation of even greater circulation falls (see figure).

The timing of this change corresponds to a fall in the size of the market for physics journals. Unless some drastic steps are taken to reverse this trend, we predict that, as far as *physics* journals are concerned, the circulation will continue to fall[3]. By the year 2000, the average subscription level of a core physics journal will be approximately 900, a fivefold drop since the late 1950s. Prices will, of course, continue to rise.



Graph showing the impact of the fall in circulation on journal prices in a sample of 160 core physics journals. Scale *A*, the actual price per standard volume, in constant (1957) dollars; *B*, the "expected" price per standard volume, in constant (1957) dollars; *C*, the average circulation per title for journals in the sample. A standard volume contains 500 standard pages[1]. The "expected" price is calculated by taking into account increases in publishing costs and falls in circulation[2]. Note the increasing divergence of the two price curves.

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1. Chomet, S. & Nejman, E. *Nature* **279**, 188 (1979).
2. Nejman E. thesis, Univ. London (1981).
3. Chomet S. & Nejman E. *J Research Commun. Stud.* 3, 185 (1981).

NEWS AND VIEWS

How large is the Universe?

Estimates of the Hubble constant have fluctuated frequently in recent decades, usually between 50 and 100 km s^{-1} per megaparsec. There is still much to be done to fix the value.

Sir Hermann Bondi once caused great consternation among an audience of physicists by declaring that because the essential parameters of the Universe were then (in the early 1950s) so poorly known, and so likely to be wrong, he proposed to ignore what passed for numerical data in a lecture on cosmology. Since then, matters have to some extent improved. Since 1974, for example, a monumental study by Sandage and Tammann (see, for example, Sandage, A.R. and Tammann, G.A., *Astrophys. J.* **194,** 559; 1974) has seemed to provide a reasonably secure basis for estimating the distance scale of the Universe. To be sure, there has been a constant rumble of discontent with the resulting estimates, so much so that most articles dealing with distant phenomena have prudently included a statement of which estimate of Hubble's constant has been used in the interpretation of data. Now the issue will have been thrown into the melting-pot again with the publication by David A. Hanes of the Anglo-Australian Telescope of an argument that the extragalatic distance scale has been overestimated by 50 per cent or so (Hanes, D.A. *Mon. Not. R. astr. Soc.* **201,** 145–170; 1982).

Nobody is to blame. The problem of laying down a distance scale within the local galaxy, which occupied the best part of a century, was a sufficient illustration of the difficulties of such exercises. As things turned out, it may be counted simply luck that there is a class of stars, the Cepheid variables, whose period of variation is a function of their luminosity, and which can thus be used as milestones within the local galaxy. Experience has so far revealed no comparably convenient method for the measurement of extragalactic distances. Instead, people like Sandage have been forced to rely on much less sharp correlations between the characteristics of some distant galaxy and its probable luminosity. But only in the past decade or so has it become clear that the statistical distance yardsticks now developed are undermined at least to some degree by the likelihood that the galaxy from which all observations are made (our own), and the Local Group of galaxies to which it belongs, are probably moving towards the centre of the Virgo cluster, probably under the influence of gravitational attraction.

What Hanes has now done is to work step by step through the analysis by which Sandage and Tammann set up their distance scale ten years ago. There is no dispute about the distance to the galaxies belonging to the Local Group — the Magellanic Clouds and the Andromeda Nebula (M33) for example — for Cepheid variables are still recognizable there and can be used as more or less certain yardsticks. Moreover, these galaxies of the Local Group serve as proving grounds for other kinds of distance yardsticks — other kinds of variable stars, red giants and the like. Hanes now points out that the crucial step in the Sandage analysis is the extension of this kind of argument to the next nearest group of galaxies, that centred on the object known as NGC2403, for this is the most distant galaxy in which Cepheid variables can still be recognized as such. The essential difficulty is that light from these variable stars may be obscured to an unknown extent by the material of which the galaxy consists, thus reducing the perceived luminosity of the objects. Hanes follows others in arguing that too little allowance has been made for obscuration, with the result that the estimate of the distance of even NGC2403 has been overestimated.

The trouble with this conclusion, forcefully advocated by Hanes, is that it applies to the other tests used by those who set out to establish extragalactic yardsticks. One of the most obvious of these is based on the assumption that the brightest (and thus the most easily distinguishable) stars in a distant galaxy are likely to be indicators of the distance of that object. The more distant a galaxy, the fainter its brightest blue stars should seem to be. The obvious pitfall is that the brightest stars in a galaxy, lying as they must on the extreme tail of some gaussian distribution, can only be indicators of distance in some statistical sense. Mercifully, extragalactic yardstick-makers are reconciled to that. Hanes's particular contribution to this discussion is to show that the brightest star criterion depends on a piece of circular reasoning. Much the same is true, says Hanes, of the other criterion used as a distance marker by Sandage and Tammann — the notion that the size of molecular hydrogen clouds associated with galaxies should statistically represent their distance from the local galaxy (which implicitly supposes that while galaxies and their associated molecular hydrogen clouds may differ from each other in their extent, the range of this variation is not *too* great). Hanes finds himself putting the reasonable proposition that the visual appearance of a distant galaxy, which is a guide to its intrinsic luminosity in some poorly known statistical sense, may be a better criterion than any other used so far. The proposition is reasonable because it depends on integrated light intensity, not the luminosity of exceptional objects.

Hanes thus comes to the conclusion that the Hubble constant is more probably larger than smaller — more like 100 km s^{-1} per megaparsec than half as much. Indeed, he neatly splits the difference between the estimate of Hubble's constant published a decade ago and that of 100 km s^{-1} per megaparsec: if the measured redshifts of the Virgo cluster galaxies are taken at their face value, Hubble's constant probably lies within 12 units of 76 km s^{-1} per megaparsec, but if the Local Group is really moving towards the Virgo cluster, Hubble's constant is probably more like 100 km s^{-1} per megaparsec. This conclusion cannot easily be disputed; Hanes's own estimated errors are, with the benefit of recent observations, narrower than his predecessors have felt able to quote. One snag, of course, is that a large Hubble's constant means a younger visible universe, which raises difficulties for those concerned with the time needed for stellar evolution. But the conflict is not sharp enough to keep people awake at nights.

So what happens next? The ideal is that somebody should find a way of correlating some property of a distant galaxy with its intrinsic luminosity that will provide a distance scale as reliable as that of the Cepheid variables within the local galaxy; that would be sheer luck. Otherwise, further refinement will depend on more painstaking statistical analysis of that which Hanes describes. This is why Hanes's own account of his proof that Sandage and Tammann were mistaken is curiously sour — it is replete with words and phrases such as "wrong", "incorrect", "untested assumption", "gives no assurances of the correctness" and so on. Hanes has every right to be proud of his latest paper on the extragalactic distance scale, for it is a thorough piece of work. But is he right to be so scornful of those whose even more careful work made possible his provocative reanalysis? Even Newton, after all, acknowledged that he had stood on the shoulders of giants.

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Trouble in the J-land

from Jan Klein and Zoltan A. Nagy

IMMUNOLOGY is not exactly noted for its lack of controversies, but the publication of the paper by Steinmetz and his colleagues[1] in this issue of *Nature* (p.35) gives the controversies a new dimension. The authors have cloned a piece of mouse chromosome containing the class II genes of the major histocompatibility complex (MHC) and report, among other things, that they cannot find at least two genes placed into this piece by immunogeneticists. Now, not finding a gene would not cause much of a stir — immunologists have been quite frivolous about putting genes into the MHC and then taking them out again — were it not for the fact that one of them is the so-called *I-J* gene (*J* for short).

The *I-J* gene was described by Murphy and his colleagues[2] in 1976. They produced an antiserum, [B10.T(6R) × B10.D2]F_1 anti-B10.AQR, which contained only antibodies specific for MHC class II antigens. Since at the time class II molecules (which provide a context for recognition of antigens by T cells; see *News and Views* **297**, 628) were believed to be present on B but not T lymphocytes, this antiserum was not expected to react with any T cells. However, when Murphy and his colleagues added it, together with complement, to a cell suspension containing suppressor T cells (cells capable of inhibiting the immune response of other cells), they discovered that the suppressive activity of these cells was abolished. When they then absorbed the antiserum by B10.A(5R) and B10.A(3R) cells, they found, to their surprise, that the former strain did and the latter did not remove the anti suppressor T cell activity. The result was surprising because the B10.A(3R) and B10.A(5R) strains had been thought to be identical in their MHC: both were derived by intra-MHC crossing-over from the same two parental strains, C57BL/10 and B10.A, and the recombinations were believed to have taken place in approximately the same region. If we designate, as was then the custom, the MHC genes of the two parents as K^b I-A^b I-B^b I-C^b S^b D^b (C57BL/10) and K^k I-A^k I-B^k I-C^d $S^d D^d$ (B10.A), then we can write the genotypes of the two recombinants as K^b I-A^b I-B^b I-C^d S^d D^d. The absorption data, however, indicated that the two recombinants were not identical and that the B10.A(5R) recombinant inherited from the B10.A parent something that the B10.A(3R) strain did not (the genotype of the B10.AQR strain, against which the antiserum was produced, is K^q I-A^k I-B^k I-C^d S^d D^d), and that this 'something' was controlled by a gene located between the *I-B* and *I-C* subregions. The authors designated this new subregion by the next available letter, *I-J*.

At the same time, another group of investigators — Tada and his colleagues[3] — reached a similar conclusion using a slightly different assay for the purportive J-specific antibodies. They reported earlier that the inhibitory activity of the suppressor T cells on other cells was mediated by a soluble factor. When they incubated the supernatant containing the factor with an antiserum similar to that used by Murphy and his colleagues, the supernatant no longer inhibited the response, presumably because the suppressor factor was removed by the J-specific antibodies.

These discoveries were quickly confirmed by other laboratories and the J-specific antibodies became firmly entrenched in the mouse MHC map and in immunologists' minds. Eventually at least one commercial company began selling anti-J sera and at least two reports appeared in the literature describing the production of J-specific monoclonal antibodies[4,5]. The original observations were then extended and the J determinants demonstrated on suppressor T cells and factors in a variety of systems. It appeared that not all suppressor T cells carried the J determinants and, in fact, the determinants were soon accepted as markers for a subpopulation of suppressor T cells. On the other hand, the determinants were also found on certain helper T cells and on macrophages. Furthermore, the J determinants were reported to stimulate mixed lymphocyte reaction, to restrict the interaction of suppressor and helper T cells, to be involved in the potentiation of immunity against syngeneic tumours, to govern the induction of immunological tolerance, as well as to do other remarkable things (see ref.6 for review). We have not counted the studies in which J-specific reagents have been used but there must be several hundred of them. In this situation, to question the orthodoxy of the *J* locus is likely to be regarded by many immunologists as a heresy of the most dangerous kind.

Yet, from the beginning, one could have had reservations about the *J* locus. For example, although J-specific antibodies had a potent effect in biological tests, they were extremely difficult to work with in serological assays. Some laboratories reported success in detecting J determinants by serological methods[7-9] but in most immunologists' hands, the cells binding the J-specific antibodies could not be visualized. The explanation offered was that the J determinants were only on a small subpopulation of lymphocytes and that the reactivity with these cells was obscured by the background reactivity of the serological assays. However, suppressor T cells can be enriched and purified and J determinants have been reported to be expressed by permanent cell lines and clones[10,11], so on these cells there should not have been any difficulty in detecting these determinants serologically. Even more disturbing was the fact that no one had succeeded in isolating the J molecules from the cell membrane. Here again, the availability of lines purportedly expressing J molecules should have provided suitable material for this isolation. Finally, there was the difficulty of the interpretation of the *J* locus. The designation *I-J* implied that *J* was a class II locus but, as we have pointed out on another occasion[12], there was no good reason for this classification. At best, the J determinants could be regarded as markers for a subpopulation of certain immune cells, but that they functioned in the same way as class II determinants do has never been satisfactorily demonstrated.

The situation with the other missing gene — the ***B*** gene — is far more clearcut than that with the *J* gene. The ***B*** gene was invoked to explain a particular pattern of responsiveness to a small number of antigens, a pattern involving the strains already mentioned, as well as another intra-MHC recombinant, B10.A(2R)[13]. No other trait could be associated with the ***I-B*** subregion and no B-specific antibodies could be produced by cross-immunization of strains purportedly differing at the ***B*** locus. Only a few immunologists felt compelled to invoke the ***B*** gene and when they did, it was only because they did not bother to think of a better explanation. That an alternative explanation did exist and that it was far more attractive than the old one was demonstrated by Baxevanis and his colleagues[14]. These investigators showed that the same pattern of responsiveness can be obtained without the postulate of the ***B*** gene but with the assumption that suppressor cells are involved in the control of the response. They then went on to accumulate convincing evidence that the alternative explanation was indeed the correct one.

Steinmetz and his colleagues have now laid the lingering apprehension about the *J* and *B* loci to rest. They have isolated the DNA from the B10.A(3R) and B10.A(5R) strains, the two strains that were used to define the *J* locus genetically, and have been able to ascertain from which parent the DNA segments around the purported *I-J* subregion have come. As expected they have found that the left-hand (centromeric) segment of these recombinants is derived from the B10 parent and the right-hand (telomeric) segment from the B10.A parent. Between these two segments they have found a region whose origin they cannot determine, and it is this region that should contain not only the *J* but also the *B* locus (in the rest of

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the DNA, the B10.A(3R) and B10.A(5R) are identical). The trouble is that this middle region is only 3.4 kilobases long and at least part of this length constitutes another gene, the well defined E_β gene. When one subtracts the minimal sequence that apparently is E_β, all that is left for the *J* and *B* genes is a sequence of less than 1,000 nucleotides. Such a sequence is, of course, far less than is required for any full-fledged gene, not to mention two such genes. It may, in fact, turn out that the B10.A(3R) and B10.A(5R) strains have identical or nearly identical DNA sequences around the site where the crossing-over occurred. In plain language, it may turn out that the *J* and *B* genes do not exist in the place where they were supposed to be. What the Steinmetz study already clearly establishes is that if the *J* and *B* genes do exist in this region, they must be quite unusual genes.

Steinmetz and his colleagues offer some graceful and some not so graceful ways out of this dilemma. The one possibility that they do not consider is that the *B* and *J* genes simply do not exist. For the *B* genes, this possibility is not difficult to accept: after the demonstration by Baxevanis and his colleagues[14] that the *B* locus is an illusion generated by a particular type of interaction between the suppressor and helper T cells, there has really not been any justification for keeping this locus in the MHC map. But the *J* gene? Is it possible that here, too, immunologists have fallen victims to some other kind of illusion? □

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Mapping the Universe

from Virginia Trimble

OUR inventory of basic kinds of objects and structures in the Universe is probably not yet complete, even on the largest scales. Thus participants in IAU Symposium 104 on 'Early Evolution of the Universe and its Present Structure'* heard of a new class of radio sources, of changes in our best estimates of irregularities in the 3K microwave background radiation, and much debate on the morphology and origin of superclusters of galaxies and the relative voids between them. The other main topic of discussion, exotic physics in the very early universe, was recently reviewed in these pages by Barrow and Turner[1].

For a quarter of a century the counting of radio sources has been telling us both about the nature of the sources and the large-scale structure of the Universe[2]. Essentially, as we count from the brightest sources towards fainter ones, we at first see more sources than expected if the objects are distributed uniformly in euclidean space, and then later we see less sources than would be predicted. J. Peacock (Royal Observatory, Edinburgh) reiterated the standard interpretation — powerful radio sources in elliptical galaxies and quasars were commoner in the past than now, but that very few turned on before the time corresponding to a redshift of about 4. P. Osmer (CTIO, Chile), A. Braccesi (University of Bologna) and A. Savage (UK Schmidt Telescope Unit) reported optical searches for quasistellar objects reinforcing this conclusion, though the last of the three groups involved has just reported a new record quasar redshift[3], $z = 3.78$ for PKS2000–330.

But, as we look to still fainter radio sources, the downward trend of number versus apparent brightness ceases, according to a new series of source counts, carried out at 1,412 MHz by R. Windhorst and others at Westerbork, and reported by H. van der Laan (University of Leiden). The most likely interpretation of this flattening in number versus flux is a new class of radio sources, which van der Laan called 'Population II radio galaxies'. They make up some 75 per cent of the sources seen at 1,412 MHz fluxes near 1 mJy, and their optical identifications are typically faint, fuzzy blue galaxies of unknown type. The radio morphology is both less tidily structured and smaller ($\lesssim$50 kpc) than that of brighter sources. The clustering properties are unknown. Van der Laan said that the new sources might be either spiral galaxies at redshifts near 0.4 or (at long last) the radio emission from so-called radio-quiet quasars, since the luminosity function is much like that of standard radio sources, except it is fainter by a factor of 1,000. The IR colours, according to Windhorst, divide into two classes, resembling those of giant elliptical radio galaxies and of less luminous spiral galaxies respectively. E. Fomalont and J. Wall presented results of an NRAO survey at 6 cm that also shows some flattening of number versus flux at the lowest flux levels surveyed and thus provides independent evidence of this (or at least some) new population of sources.

Looking for structure in the 3K background radiation is necessarily a less ancient activity than counting radio sources, though it is coeval with the discovery of the radiation itself[4]. N. Parijskij (Special Astrophysical Observatory), F. Melchiorri (Rome), D. Wilkinson (Princeton University), K. Kellermann (NRAO) and A. Lazenby (Jodrell Bank) reported on searches for anisotropies on various size scales and at various wavelengths carried out in the USSR, Italy, the US and Great Britain. The main conclusions follow.

First, the dipole anisotropy, attributable to our motion relative to the particles that last scattered the radiation, is here to stay. Though there is still some discrepancy between the Berkeley and Princeton results (owing, apparently, to calibration errors), we can say that the Sun is moving at 372 ± 25 km s^{-1} towards right ascension 11.2 h, $\delta = -6°$. This translates into a ~ 600 km s^{-1} velocity for the Local Group of galaxies, aimed about 30° away from the Virgo cluster, and so is at least roughly consistent with the result reported by J. Mould (Kitt Peak) from optical observations of galaxies of a few hundred km s^{-1} infall towards Virgo plus ~ 180 km s^{-1} rotation around it. The dipole anisotropy shows a small variation with a period of one year. Copernicus was right!

Second, the quadrupole anisotropy reported by two groups[5,6] has gone away and was apparently an artefact of improper subtraction of the contribution from continuum radiation by our own Galaxy. It has left behind a bright spot outside the galactic plane in the 0.6–2 mm data of the Rome group[7] but not in the longer-wavelength data from Berkeley and Princeton. Wilkinson suggested the hot spot might be thermal radiation by a large galactic dust cloud, though Melchiorri suspects it may have cosmological significance.

Third, on smaller scales, there continue to be mostly upper limits, $\Delta T/T \leq 3 \times 10^{-4}$ for 1′ to 1° scales from the NRAO and Jodrell surveys and $\leq 3\text{–}5 \times 10^{-5}$ on similar scales from the Rattan 600 (USSR) survey. G. de Zotti (University of Padova) suggested that the second of these limits could not be correct, because known radio sources alone should contribute larger fluctuations; and the American and British observers expressed audible curiosity about how the measurements were made. Parijskij's response was that the Rattan telescope is simply more suitable than most others for work on extended emission. Even the higher limits are already serious constraints on our models for cluster/galaxy formation because they pertain to the angular size of clusters at the

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largest redshifts at which adiabatic perturbations in the matter density can begin to grow — we must have reasonable lumpiness then to get the lumpiness we see now. Ways out of this dilemma include, according to E. Salpeter (Cornell University), B. Jones (University of Paris), S. Bonometto (University of Padova) and N. Kaiser (University of Cambridge), an initial spectrum of fluctuations whose shape was not a power law; re-ionization at $z > 10$ to smooth out the lumps in the radiation but not the matter; galaxy and cluster formation by some process that makes the necessary lumps after recombination, as suggested by Ostriker and Cowie[8]; massive neutrinos, which can begin to clump as much as they want without distorting the radiation once they stop interacting with the photons (which happens much sooner for them than for ordinary matter); or non-adiabatic perturbations (which are, however, not permitted by modern grand unified theories[1]).

On all scales, we cannot hope to do much better than present accuracies and limits, which, according to Wilkinson, are within a factor of 3–4 of noise levels.

The study of superclusters (clusters of clusters of galaxies) and the voids between them is the oldest of all these activities; for, as J. Oort (University of Leiden) pointed out, their existence and main properties are already implicit in the Shapley–Ames catalogue[9] of 1932. There is general agreement on the existence of structures of up to 100–150 Mpc, separated by about twice that amount. The structures are at least somewhat elongated, with crossing times on the long axis greater than or approximating the age of the Universe and 10^{15}–10^{17} the mass of the Sun. And the density contrast between supercluster and void is sufficient (at least factors of 2–4) that we see some deceleration of the general Hubble expansion of the Universe at the edges. The chief observational items of dispute are (1) just how empty are the voids, (2) whether the typical supercluster shape is an ellipsoid, a pancake, or a string, and (3) the extent to which the superclusters join up into a network or cell structure. These bear directly on the chief theoretical question of how the superclusters formed. The competing models present explanations in terms of gravitational clustering of smaller-scale structures or fragmentation within pancake-like gas sheets formed by nonlinear growth of adiabatic perturbations.

Both models can make superclusters with roughly the correct size and correct first- and second-order correlation functions[10]. But the pancake models should result in emptier voids, more stringy features and more networking than the clustering models[14,15].

Looking into the voids, S. Shectman (Mt Wilson) reported an extension of the Kirshner *et al.*[11] redshift survey that confirms the existence of a sphere 120 Mpc across (for $H_0 = 50\,\mathrm{km\,s^{-1}\,Mpc^{-1}}$) centred at right ascension 15 h, $\delta = +50°$, $V_r = 15{,}000$ km s^{-1} in which the density of galaxies is at most one quarter that of the surroundings. In fact, they find no galaxies at all in that sphere, though there are apparently modest numbers of emission line galaxies[12,13] in adjacent zones previously thought to be empty.

As far as shapes of superclusters go, our own Virgo one is a 6:3:1 ellipsoid according to R. Tully (Hawaii). But it is apparently far from typical. J. Einasto (Tartu) and F. Shandaring (Institute of Applied Mathematics, Moscow) reported applications of a new (to most astronomers!) technique, called percolation theory[16], which is particularly suited to determining topology (degree of connectedness) of structures, rather than just the amount of clustering. The primary conclusions are that the basic structures are strings of clusters and galaxies with a filling factor of about 1 per cent; that essentially all the strings are connected into a network; that the luminosity contrast between strings and voids is at least 10^3, though there are small strings within voids like the one of Kirshner *et al.*[11]; and that no one model copes simultaneously with all the data.

Finally, R.H. Miller (ESO and University of Chicago) showed cinematically the results of assorted numerical clustering experiments, some of which seemed to the eye to have produced much the same sorts of filaments and cells as seen in the distribution of real galaxies, though this has not yet been tested by percolation theory or anything similar. But he left us with the important thought that, just because simulation of a process produces something that looks like the real world, this does not necessarily mean that nature did it that way! □

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Air pollutants act in combination

from J.N.B. Bell

NEW work from the University of Lancaster reported in this issue of *Nature* (p.55) highlights the importance of the combined effects of NO_2 and SO_2 — Britain's two most widespread air pollutants — in reducing the growth of grasses and trees. The work demonstrates the complexity of growth responses to SO_2/NO_2 mixtures and the danger of attempting to predict injury in the field on the basis of the single harvest fumigation experiments which are invariably employed as the basis for developing air-quality criteria.

Whitmore and Freer-Smith[1] fumigated *Poa pratensis* (meadow grass) and cuttings or seedlings of six tree species, *Malus domestica* (apple), *Betula pendula* (silver birch), *Betula pubescens* (common birch), *Populus nigra* (black poplar), *Alnus incarna* (grey alder) and *Tilia cordata* (small-leaved lime), for up to eleven months in outdoor glass chambers, in the presence of SO_2, NO_2, and SO_2 and NO_2 at concentrations (0.062 p.p.m.) encountered in some urban regions. Growth rates were compared with those of plants in clean air.

When *P. pratensis* was fumigated overwinter, the three gas treatments produced markedly different results between November and February — SO_2 had relatively little effect, NO_2 stimulated growth, and the mixture produced a substantial fall in dry weight. The greater than additive effect of the gas mixture subsequently disappeared, and reductions in growth became apparent in all treatments, although they recovered to the same level as the controls by June. A shorter summertime fumigation of the trees produced a greater than additive effect with SO_2 and NO_2 in four out of the six species.

That the air of suburban areas of British cities contains pollutants that can cause large reductions in the growth of grass species has been demonstrated by comparing the growth of plants grown in chambers ventilated with ambient or clean air[2-4]. Such effects may occur when the mean SO_2 level is much lower (0.011–0.026 p.p.m.) than that which reduces plant growth in fumigation experiments (≥0.038 p.p.m.)[5], suggesting that the fumigation design does not take into account the

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presence of other pollutants and the continuous fluctuation of SO_2 in ambient air[6].

Relatively recently, fumigation experiments have begun to be carried out using continuously fluctuating pollution levels. Two recent reports, however, indicate that the occurrence of peak concentrations of SO_2 may not be as important as has been suggested. Fumigations of ***Phleum pratense*** (timothy) with 0.06 p.p.m. SO_2 for 41 days produced the same effects on growth, irrespective of whether the pollutant was administered at a constant level, or with peak concentrations of 0.4 and 0.8 p.p.m. respectively[7]. Similarly, when ***Pinus sylvestris*** (Scots pine) seedlings were fumigated over 650 days with 0.038 p.p.m. SO_2, given either at a constant level, or as four treatments comprising factorial combinations of high (0.281 p.p.m.) and low (0.113 p.p.m.) hourly peaks over short (5 h) and long (21 h) periods, the constant treatment resulted in a 14 per cent reduction in dry weight increment, and peak concentrations did not produce any further growth reduction, unless they were of 21 hour duration[8].

It thus seems likely that the presence of other pollutants, in addition to SO_2, is a major factor in reducing plant growth in cities. High-temperature combustion processes produce abundant quantities of nitrogen oxides (NO_x), which have hitherto been little studied, expect for their role in the formation of photochemical oxidant smogs. NO_x is emitted into the atmosphere mainly in the form of NO, irrespective of whether the source is motor-vehicle exhaust or coal/oil-fired power stations, but is rapidly oxidized to NO_2. The limited data available show that mean NO_x levels are equal or greater (v/v) than mean SO_2 concentrations in both urban and rural areas. A SO_2/NO_x ratio of 1:1.5 has been recorded for central London[9] and it has been suggested[10] that approximately 11 per cent of rural land in western Europe may be exposed to SO_2 and NO_2 concentrations of > 0.038 and > 0.042 p.p.m. respectively. Clearly there is a need for the incorporation of NO_2 (and possibly NO) into fumigation experiments designed to investigate SO_2 effects on plants. Both additive and more than additive responses have been observed for SO_2/NO_2 mixtures on different grass species[11] but the work of Whitmore and Freer-Smith demonstrates for the first time that such effects are markedly influenced by season.

Considerable difficulty has been experienced in determining thresholds for long-term SO_2 injury to grass species[12]. Interactions between changing environmental conditions and SO_2 effects have been suggested as the main factor obscuring a clear dose–response relationship. Whitmore and Freer-Smith have identified another important factor, by showing that SO_2, NO_2 and their mixture have very different effects if the fumigation is terminated at different times of the year. Their work demonstrates the need for further such long-term experiments, with harvesting at intervals and, if possible, extension over several growing seasons in order to determine the net result of the positive and negative growth effects of the pollutants. □

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Transformation and gene organization in *Drosophila*

from Michael Ashburner

THE highlight of Crete III, the third EMBO-sponsored workshop* on the molecular biology of *Drosophila*, was, without doubt, the announcement by G. Rubin and A. Spradling (Carnegie Institution, Baltimore) that they had successfully transformed *Drosophila* with exogenous DNA — results that have just made their appearance in *Science* (October 22, p.348). Moreover, D. Ish-Horowicz (Imperial Cancer Research Fund, London) reported that, in collaboration with J. Sang's group (University of Sussex), the transformation of *Drosophila* tissue culture cells with DNA had also been achieved.

The flies were transformed by the injection of exogenous DNA into embryos using the transposable P element as a vector. The P elements of *Drosophila* are repetitive genetic elements found only in certain strains (so-called 'P' strains) and absent from other ('M') strains. In hybrids between a P strain and an M strain, the P elements contributed by the P parent may become highly unstable (especially if the cross was between a P father and an M mother), to produce the syndrome of hybrid dysgenesis (see *News and Views* **299**, 676; 1982).

Rubin and Spradling constructed a vector that contained a wild-type *rosy* gene sequence within a P element sequence. Flies mutant for *rosy* have brownish eyes, since they lack the xanthine dehydrogenase (XDH) needed to synthesize the normal red eye pigments. *rosy* is particularly useful for this sort of experiment as only a few per cent of normal XDH activity is necessary to ensure a wild-type eye colour and the gene need not be active in the 'correct' tissue (or, perhaps, even at the 'right' stage of development) for a wild-type eye colour to result. Two successful methods of transformation were described. In the first, rosy-P plasmid was injected into embryos that were both mutant for *rosy* and carried their own integrated P elements on a dysgenic background. In the other, the rosy-P plasmid was co-injected with a cloned intact P element into non-dysgenic, and non-P-carrying, embryos. The introduced *rosy* genes integrated into the genomes of their hosts and at least one was active enough to ensure a wild-type eye colour.

The only other genes which have, so far, been used to transform flies are those coding for the egg-shell proteins. These are of particular interest for they amplify during normal development in the ovarian follicle, the tissue that secretes the egg shell. Spradling discussed the results of transformation with two different egg-shell protein gene fragments. In the transformed fly strains, three to four copies were integrated at various chromosome sites. One of these fragments amplifies according to its normal developmental schedule, despite its unusual location in the genome. This promises to be a powerful method for the study of those sequences responsible for the normal tissue-specific amplification of these genes.

Transformation was not, of course, the sole topic of discussion in Crete. Studies on the molecular organization of genes in *Drosophila* now seem to indicate that there are several different discrete categories of gene. The simplest are those present in a single copy. Only two have been reasonably well characterized — *rosy* (coding for XDH; W. Bender, Harvard Medical School) and the gene for alcohol dehydrogenase (T. Benyajati, Frederick

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*The 3rd Workshop on 'The Molecular Biology and Developmental Genetics of Drosophila' was held at Kolymbari, Crete, on 21–26 June 1982 and was sponsored by EMBO and the University of Crete.

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Cancer Institute, Maryland; M. Ashburner and colleagues, University of Cambridge).

Yet even these genes may not be that 'simple'. At *Adh*, for example (Benyajati; N. Spoerel, University of Cambridge), transcription occurs from two quite different promoters in different tissues and at different stages of development.

Several 'complex' genes — complex in terms of their genetic organization and their developmental phenotypes — have been identified by *Drosophila* geneticists. Some of them are now cloned: *bithorax* (Bender; M. Akam, University of Cambridge; R. Saint, Stanford University), *Notch* (M. Young, Rockefeller; S. Artavanis-Tsakonis, Yale University), *scute* (J. Modolell, University of Madrid) and *rudimentary* (K. Louis, Princeton University) are examples. All are very large (*scute* is spread over at least 60 kilobases) and all produce a bewildering variety of developmentally regulated transcripts — at least four have been found at *scute*, each with quite different developmental specificities.

A third class of gene may be called 'clustered multicopy'. The copy number is low, from two to less than ten, the copies are all contained within a relatively short stretch of DNA (few tens of kilobases) and the different copies have often diverged so that they code for different, but functionally related, proteins. Several examples are known: the four genes coding for the small heat-shock proteins (E. Craig, University of Wisconsin-Madison), three genes coding for salivary gland glue proteins (E. Meyerowitz, Caltech) and the two major chorion protein gene clusters (Spradling; F.C. Kafatos, Harvard University) are well worked out. Presumably the members of a cluster are related by descent. However, not only are they often transcribed from different strands of the DNA but the extent of divergence since their presumed duplication may be considerable. Of the three different glue genes, one codes for a protein of 284 residues and the other two for much smaller proteins (about 50 amino acids). The large protein consists of four domains — a hydrophobic leader, 44 amino acids very high in threonine, 37 copies of a five-residue repeat and a C-terminal domain of 50 residues, very high in cysteine. The small proteins 'simply' lack the internal two domains! (Meyerowitz).

A fourth class of gene is the 'dispersed multicopy'. Again copy number is low, less than ten, but the copies are dispersed around the genome. If there are rules governing their location we do not know them yet. Examples are the actin genes (N. Davidson, Caltech), the genes coding for the larval serum proteins (four genes; D. Roberts, University of Oxford) and the *Hsp68* and *Hsp70* heat-shock genes and their cognates (Craig). Again, studies of the different copies suggest that they have evolved by duplication. Presumably (but not necessarily) they have been dispersed subsequently by a transposition process. Why some genes should be organized in small clusters and others dispersed is not yet clear. Of course some genes, for example, *Hsp 70*, share characteristics with both classes, being dispersed clusters. Finally, there is the class of tandemly repeated, high-copy number genes such as those coding for the rRNAs and the histones.

A fascinating family of transposable elements (TE) has been discovered by Bender. Called 'gypsy', they are often associated with spontaneous mutations that share the property that they can be suppressed by a recessive mutant allele of *su(Hw)*. Presumably gypsy acts at a distance, since revertants of some gypsy-associated *bithorax* mutations are only partial losses of the gypsy element itself. The suppressor does not act by causing the element to excise. Of quite a different order from these elements is G. Ising's (University of Lund) TE, which is vast (» 100 kb) and carries at least two enes (*white* and *roughest*). R. Paro (University of Basel) has cloned some examples of this TE: its transposable property apparently results from long 'fold-back' sequences at its ends. This 'FB-NOF' sequence also occurs elsewhere in the genome, for example the unstable *white-crimson* mutation is associated with this element.

Although molecular biology dominated the meeting it must not be thought that the gene-jockeys had it all their own way. An elegant demonstration of the error of inferring too much from the phenotype of mutations in a complex eukaryote such as *Drosophila* came with B. Baker's (La Jolla) study of mitosis in a random selection of conditional lethal mutations. Many had been selected for some 'interesting' developmental phenotype, for example an absence of imaginal disks. A suprisingly high proportion of the mutations turn out, however, to disrupt mitosis and some are very dramatic — accumulating over 1,000 chromosomes per nucleus. All diploid cells are affected. Although the mutants were originally called 'diskless' because this is the most prominent feature of their phenotype, they are clearly not 'imaginal disks' mutations in anything but a rather trivial sense.

The vigour of modern *Drosophila* biology was evident at Crete. The discovery of new molecular and genetic techniques cannot but fail to keep *Drosophila* research in the forefront of modern biology. This, and the patronage of the Crete meetings by the Bishop of Kastelli Kissamou, augurs well for Crete IV. □

Food — the best medicine

from Ben J. Miflin

EVERY so often society rediscovers the obvious. Currently many sections of society from Wall Street to Westminster are rediscovering plants and man's dependence on them. The waves of enthusiasm emanating from the new converts have led to the founding of numerous companies in the US and may yet be strong enough to push the British Technology Group into launching Celltech's country cousin (*Nature* **297**, 5; 1982). But where to sail? What to do? What to improve? Many think-tanks have pondered these questions and the CIBA Foundation* has recently broken its long-standing tradition of medically based symposia to address the topic of 'Better Crops for Food'.

First, define 'better', define 'food'! The answer depends on where you are standing and on how carefully you look at a particular agricultural system (C. Spedding, University of Reading). An apparent improvement may work in one context, be irrelevant in another and be absolutely disastrous in a third. Only if there is some appreciation of how the different systems work is there a chance of predicting, usually on the basis of modelling, what the effect of a development might be or which changes might be desirable.

Man has catalogued only a proportion of the world's plant species and he depends on 15 or so for the bulk of his agriculture (E. Bell, Royal Botanic Gardens, Kew). Many plant species are being lost irretrievably and these include reservoirs of germplasm relevant to our crops. However, what and how we should conserve is a matter of controversy. One answer may be the application of cryogenic techniques for storing plant tissue cultures (K. Dougall, University of Tennessee), but predicting what may be useful in the future is uncertain. New crops may emerge but the conservatism of agricultural and cultural practices limits likely crops to those which are already grown to some extent in marginal areas. The winged bean (*Psophocarpus tetragonolobus*) is one likely candidate; it seems to have many virtues including protein-rich tap roots and metre-long pods that can be eaten green or dried (N. Haq, University of South-

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* The Ciba Foundation meeting on 'Better Crops for Food' was held 14–16 September. The proceedings will be published as Ciba Symposium 97 (Pitman Books).

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ampton; S. Karikari, Ahmadu Bello University, Nigeria). Traditional intercropping systems practised in developing countries may also be a more appropriate guide to future cropping systems than western monoculture, as studies at ICRISAT have shown (R. Willey, University of Reading). The advantages may not be restricted solely to the tropics as mixed cropping of cultivars of barley and wheat, developed to combat disease, may yield more even in the absence of disease (P. Day, Plant Breeding Institute, Cambridge, quoting results of Wolfe).

The aims in breeding better crops are usually maximum yield (although maximum financial return may be better), disease resistance and nutritional quality. Improved yield has generally resulted from improved harvest index rather than from changes in carbon dioxide fixation rates. Nutritional quality in legume seed depends on many components (D. Boulter, University of Durham) and genotypes of soybeans lacking some of the undesirables ones have been found (T. Hymowitz, University of Illinois). Whether it is worth incorporating these traits into commercial cultivars depends on the rest of the diet, how the beans are cooked and whether the components confer pest or disease resistance on the seed.

Increases in yield, particularly of cereals, has resulted equally from improved cultivars and improved agronomic practice. Classical agronomic research started in the UK at Rothamsted with the aim of improving soils to fit the needs of the crops available. The approach may be curtailed in the future by the lack of energy to produce fertilizers (E. Bell), although others (R. Hardy, Du Pont, Wilmington; B. Miflin, Rothamsted Experimental Station) argued that this was unlikely as these inputs are a minor part of fuel use and often produce a positive energy return. Replacement of nitrogen fertilizer for cereals, by introduction into them of nitrogen fixation genes or by the use of associated symbioses, is unlikely other than in the very long term. However, rhizosphere microorganisms, including *Azospirillum*, may have an important role in enhancing nutrient uptake by crops (R. Hardy). Crops can also be tailored for the soils we have, whether they are naturally high in aluminium as in Brazil or made saline by continual irrigation as in California (E. Epstein, University of California, Davis). Energy to process crops into food may be the major input in both developed and developing countries; in the former it is often unnecessarily excessive, in the latter it can be a matter of life or death as when cooking inactives toxins. Developing countries are also suffering an energy crisis due to the destruction of trees which, for many reasons, are important components of agricultural systems (P. Nair, International Council for Research in Agroforestry, Cambridge).

Thus there is no lack of problems. But what of new tools to tackle them? The microchip makes computing on the farm possible and many systems are being developed to maximize efficient use of inputs; one such — EPIPRE — was described by J. Zadoks (Wageningen Agricultural University, The Netherlands). This predicts the optimum amount and timing of the use of crop-protection chemicals on a field-by-field basis. The result is mostly to reduce the amounts applied, thus reducing cost and preventing pollution — still further reductions will come with improvements such as electrostatic spraying. Practitioners of genetic engineering techniques, despite the current enthusiasm, generally do not see the dramatic production of 'super-plants' in the near future. What these techniques will do is improve our understanding of plant genetics and provide technical short-cuts for breeders. Flavell (Plant Breeding Institute, Cambridge) decribed two such applications for recombinant DNA techniques: for rapid measurement of genetic diversity in order to recognize desirable types and for a quick and sensitive assay to detect plant viruses. Each technique could shorten the long time required at present to breed new varieties. 'Intermediate' technologies which may be of short-term significance are those which produce genetic diversity, such as 'somaclonal' variation. This term, coined by W. Scowcroft (CSIRO, Canberra) describes the variation released during de- and re-differentiation of plants through protoplast and tissue culture techniques which is currently being assessed for commercial promise in several crops. Further down the line are protoplast techniques, including somatic cell fusion, which have the promise of transfer of desirable traits across the barriers of sexual incompatibilities (O. Schieder, Max Planck Institute für Zucktungsforschung, Cologne).

By way of conclusion, Spedding's quotation from Poul Andersson seems appropriate "I have never encountered any problem however complicated which, when looked at in the proper way, did not become still more complicated". In producing more food to meet the demand of the world's rapidly rising population there is no one problem, no one answer, only an almost infinite range of possibilities. In these circumstances research priorities should be diverse enough to provide various alternative solutions to the most likely crises. Evidence that this is the route to success comes from plants themselves, as E. Bingham (University of Wisconsin-Madison) elegantly showed. Lucerne cultivars bred for maximum heterozygosity have maximum vigour; dependence on a narrow (genetic) base leads to weakness and depression. □

100 years ago

A Curious Halo

THERE appeared in Nature, vol. xxvi. pp.268, 293, two articles headed "A Curious Halo," which reminded me of an analogous and still more curious phenomenon occurring sometimes here in China, during the hot season. I beg to hand you a few lines on that subject, from the *Monthly Bulletin* of the Zi-ka-wei Observatory for August, 1877.

"A phenomenon to which I wish to call the attention of meteorologists was obseved many times during that month (August), as also in July. It does not seem to take place in Europe, and I am inclined to think that it cannot occur except with an atmosphere over-charged with aqueous vapour, as it is the case with us in July and August. In the evening, just after sunset, or in the morning even long before sunrise, no matter what the direction of the wind and the barometric pressure may be, provided the day or night were very warm, *bands* of a tint varying from the faintest to the deepest *blue* are seen to appear upon the whitish or roseate vault of heaven. They usually are first seen in the east at evening and in the west at morning time, seemingly radiating from a common centre diametrically opposite the sun's position. At other times they emerge from the very position of the sun, or from both points at once, the interval being either free from bands or completely encircled by them.

"Last year (1876), on the morning of September 4, I enjoyed a most interesting sight. It was about 5 a.m., the moon, then on her nineteenth day, was above the western horizon, and just being partially eclipsed; now from her bright disc, as from a radiating centre, shot out a number of those bands or blue beams; they traversed the whole expanse of the sky, and seemed to converge towards a point whose situation in the east below the horizon corresponded with that of the moon in the west above the horizon.

"The bands or shoots are more or less numerous, bright, and persistent; some have been observed in the evening, fortyfive minutes after sunset, and in September, 1876, I saw them appear with the first break of day. They are evidently movable in the sky, and there is no doubt that they are due to comuli floating about the horizon. below or above, through which the light of the sun is sifted and split; they are, in fact, nothing else than the twilight. According as the clouds before the sun are more or less compact or loose, the bands may be blue, white, or red. More than once also have I seen the sky half white and half blue, the separation of the two colours being plainly perceivable, and Venus shining brilliantly in the blue sky close to that limit, whilst it would probably have been almost invisible through the milky sky hard by."

Any one who gazes for the first time at this beautiful phenomenon cannot help wondering and acknowledging it to be greatly different from anything to be seen elsewhere. It is quite certain that the phenomenon is due to the atmospheric vapour, but I am rather at a los to give a more satisfactory explanation. The dispersion of the direct rays of the sun *into* the minute drops resulting from a partial but wide-spreading condensation of the aqueous vapour in the upper strata of the air, might account for evening time. Besides, the interposition of a light cloud in the way of the sun's rays does not impair the transparency of the drops, and the blue sky may be visible. Now, in the morning and evening the rays of the sun are almost parallel with the horizon; they traverse the whole expanse of the sky, and their apparent convergence on the both sides is only due to the same optical illusion which shows us the two rails of a railway track or the walls of a tunnel as converging.

MARC DECHEVRENS

Zi-ka-Wei Observatory, near Shanghai,

From *Nature* 27, 30, 9 November 1882.

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Fire deaths and toxic gases

from Barbara Chernov Levin

AMONG the industrialized countries which record statistics on fires, the United States has the most fires per capita. The rate of fire death in the United States is almost twice the international average and is surpassed only by Canada[1]. In 1979, approximately 7,800 people died and another 31,000 were reported injured[2]. Direct dollar loss was greater than $5 billion, but when indirect costs (meeting fire codes, installing fire protection systems, supporting fire departments and insuring property) are included in this calculation, the annual cost of fires in the United States is estimated at more than $20 billion.

Large fires receive the most public attention and media coverage; for example, three hotel fires between November 1980 and May 1981 were the cause of 119 fire deaths and more than 1,000 injuries. Residential fires, however, continue to be the cause of most fire deaths and injuries. Approximately 80 per cent of these fire deaths are not due to burns, but are attributed to inhalation of toxic smoke and hot gases[3]. This information has prompted the fire community, materials manufacturers and building code officials to pose the following questions: do some building materials and furnishings produce combustion products that are much more toxic than others, and how can the occupants of buildings be protected from unnecessary risk in case of a fire? The answers to these questions are not simple and involve the evaluation of the total toxic hazard generated by a fire.

The potential for generating toxic gas from burning materials has long been recognized; for example, in the 1929 Cleveland Clinic fire, 123 people were killed by the decomposition products of nitrocellulose X-ray film[4]. Only recently, however, has the fire research community become acutely aware and concerned about the problem of the toxicity of combustion products and the possibility that toxic gases other than carbon monoxide (still a primary toxicant in fires) may have an important role. Autopsy data show a sample of fire victims with less than lethal amounts of carbon monoxide in their blood[3,5,6]. Other factors may have been involved, however, as some of the victims had pre-existing heart disease and/or alcoholic intoxication.

Laboratory research studies also suggest a role for toxicants other than carbon monoxide. A highly toxic bicyclic phosphate compound produced during the thermal decomposition of a laboratory-formulated rigid flame-retarded polyurethane foam was identified by the University of Utah[7] and suggests the need for an animal testing system — conventional chemical techniques would have failed to detect such an unusual toxic product. Moreover, scientists at the National Bureau of Standards have found that carbon monoxide concentrations measured at the LC$_{50}$ of twelve thermally decomposed materials were 60–4,400 p.p.m., whereas the LC$_{50}$ of pure carbon monoxide was 5,000 p.p.m. The LC$_{50}$ here measures the amount of material necessary to kill 50 per cent of the rats exposed for 30 min. The death of the rats at less than the LC$_{50}$ of pure carbon monoxide indicated that additional toxic gases or other factors (for example, heat, lowered oxygen concentrations) were contributing to the cause of death. Many of the exposed rats also died during a 14 day post-exposure observation period, an occurrence which cannot be directly attributed to carbon monoxide poisoning.

Some of these thermally decomposed materials also produced the potent toxicants hydrogen cyanide and/or hydrogen chloride as well as carbon monoxide. Chemical analysis has detected many combustion products in addition to the frequently examined toxicants — carbon monoxide, carbon dioxide, hydrogen chloride, other halogens, oxides of nitrogen and sulphur, and ammonia. Scientists at the Fire Research Station in England have identified 20 chemical species from the thermal decomposition of polyacrylonitrile and 30 combustion products from polypropylene[5]. Michal *et al.* have identified 71 compounds from the combustion of polyethylene and 55 compounds from polypropylene[9]. Wood generates at least as many products[10]. The relative contribution of one or various combinations of these combustion products to animal toxicity is so far unknown and would require a major research effort to decipher.

The current awareness of the importance of combustion product toxicity is apparent from two recent symposia (Annual Society of Toxicology meeting, February 1982; American Chemical Society national meeting, March 1982), and by the newly convened combustion product toxicity commitees of the US National Fire Protection Association and of the US National Institute of Building Sciences. Recently, three different animal-based test methods designed to assess the acute inhalation toxicity of combustion products were proposed to the Committee on Fire Standards of the American Society for Testing and Materials for their consideration. Worldwide concern about the toxicity of combustion products is indicated by increased activity in a Subcommittee on Toxic Hazards of the Fire Test Committee of the International Organization for Standardization and the development of separate tests in Germany, France, Japan and Belgium.

All these tests provide information on the acute toxicity resulting from the inhalation of combustion products, but each test is performed in different laboratory decomposition conditions. The tests do not address the problem of the total toxic hazard which a fire produces or the contribution of a material (or product in its actual end use) to that hazard under a 'real fire' situation. Nonetheless, some building code officials and state regulators are anxious to evaluate the combustion product toxicity of materials and are examining these test methods with that end in mind. It is important, however, that the acute toxicity (the harmful effects measured by the response of laboratory test animals to a single short exposure to combustion products generated by the thermal degradation of materials in specified conditions) be distinguished from the toxic hazard (the materials's properties and the environmental conditions which increase the probability that a toxic atmosphere will occur and an injury will result). Since any one fire is a dynamic, changing condition and no two fires are alike, no one test can duplicate all 'real fires'. Typical fire degradation modes (smouldering, radiant heat, non-flaming and flaming) can be reproduced in the laboratory, however, and materials can be tested in these conditions. The resultant laboratory toxicity data and other needed information, such as the projected end use of the product, the quantity of material present, its ignition and combustion properties, ventilation conditions, and the density and sensory-irritant properties of the smoke, may then be related to the total toxic hazard. Many of the investigators involved in this area of research are now turning their talents towards the more challenging field of toxic hazard analysis. ☐

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Erratum

In the *News and Views* article 'Promiscuous DNA — chloroplast genes inside plant mitochondria' by John Ellis (*Nature* **299**, 678; 1982), the word 'within' in the sentence 'Most polypeptides found in the bioenergetic organelles are encoded and synthesized within the organelles themselves' should be replaced by the word 'outside'.

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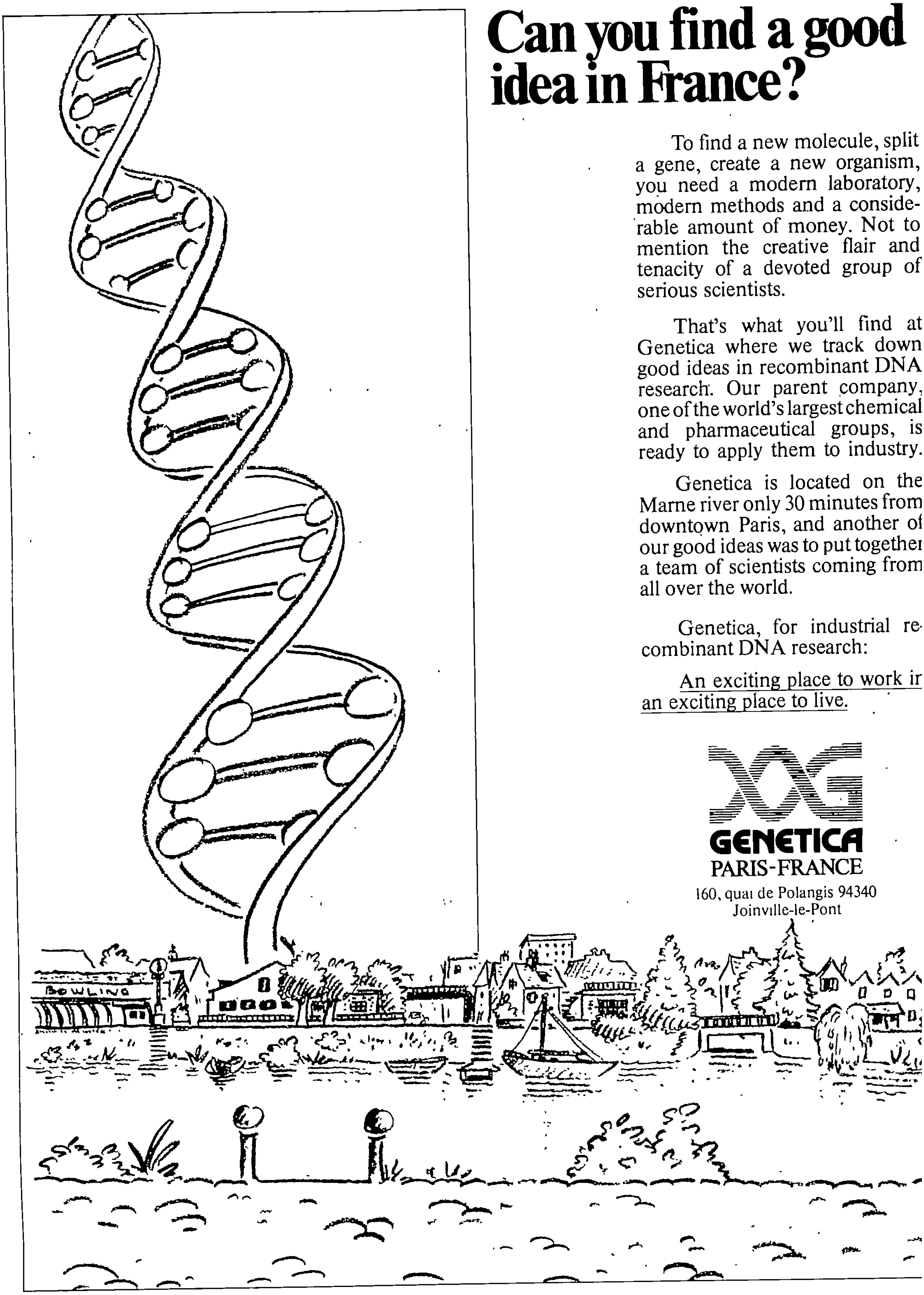

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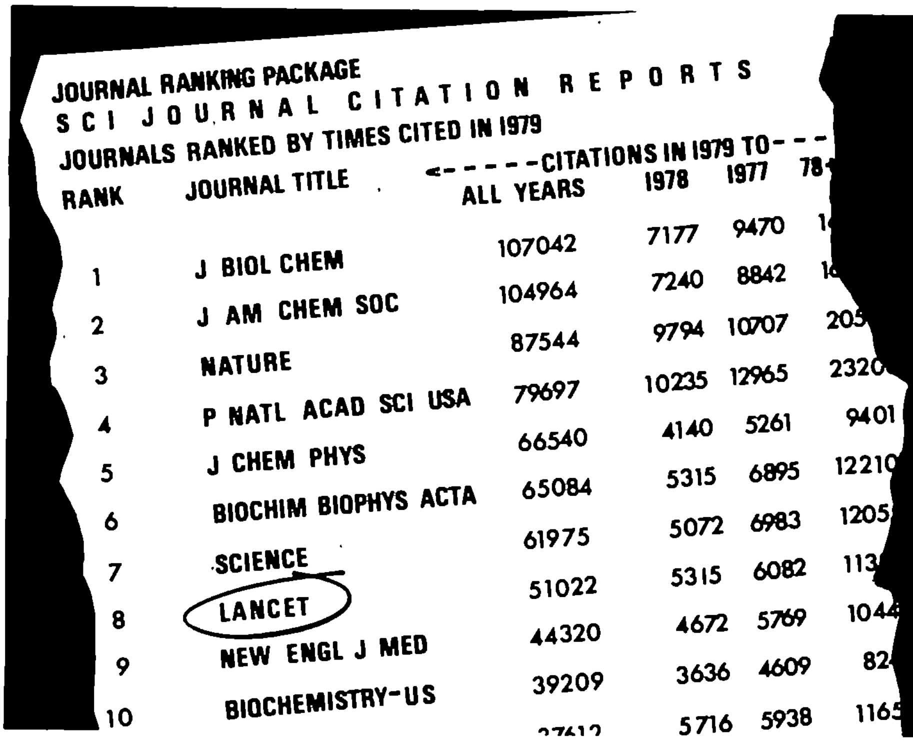

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REVIEW ARTICLE

Genetic and molecular mechanisms of viral pathogenesis: implications for prevention and treatment

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The pathogenesis of infection of mice by the mammalian reoviruses involves several discrete steps. Each of the three viral outer capsid proteins has a highly distinct and specialized role: one protein (σ1) binds to cell surface receptors; a second protein (μ1C) determines the capacity for viral growth at mucosal surfaces; and the third protein (σ3) is responsible for inhibiting cell macromolecular synthesis. A detailed picture of the molecular basis of reovirus virulence and attenuation is now emerging.

TECHNOLOGICAL developments over the past decade have enhanced our understanding of the biochemical basis of heredity and have led to an increasing understanding of the organization and regulation of eukaryotic genes. Although microbial systems have provided many of the models for these basic studies, the complex nature of the interactions between microbial agents and eukaryotic hosts that occur during the infectious process have hindered the deciphering of the molecular mechanisms involved in infectious diseases[1]. Thus, while many elegant studies have been performed on the descriptive aspects of the pathogenesis of infectious diseases, little is known about the precise molecular events responsible[2]. Recently, increased knowledge of the functions of individual components of viruses and bacteria and their interactions in the ultimate production of infection have begun to allow research directed towards defining molecular mechanisms of pathogenesis[1,3,4].

Here we review studies using the mammalian reoviruses as a model of microbial pathogenesis. We shall describe the role of various viral components in the pathogenesis of reoviral infection of young mice, and illustrate several specific genetic and immunological manipulations which yield attenuated viruses. One of the major goals of studying viral pathogenesis with model systems such as the reoviruses is to understand the processes of attenuation and immunization of microbial pathogens and to develop new methods for the prevention and treatment of infectious diseases.

Reovirus structure and morphology

Genome structure. The mammalian reoviruses include three serotypes[5]. The viral genome consists of three size classes of segmented double-stranded RNA (dsRNA): large (L), medium (M) and small (S), each of which consists of unique segments[5]. L contains three genome segments (L1–L3) with molecular weights of ~ 2.3–2.5×10^6; M consists of three genome segments (M1–M3) with molecular weights of ~ 1.4–1.6×10^6; S has four genome segments (S1–S4) with molecular weights of ~ 0.6–0.9×10^6. Each of the 10 genome segments represents a single gene which is transcribed and translated into a unique mRNA molecule and a primary polypeptide, respectively[6–9].

Virion polypeptides. Solubilization and separation by polyacrylamide gel electrophoresis of the proteins in cells infected with reovirus yields three size classes: large or λ (λ1, λ2, λ3), middle or μ (μ1/μ1C, μ2, μNS), and small or σ (σ1, σ2, σNS, σ3)[7]. Nine of the polypeptides are present in purified virions (λ1, λ2, λ3, μ1/μ1C, μ2, σ1, σ2 and σ3) while two are found exclusively in the cytoplasm (μNS, σNS)[8,9]. The μ1C polypeptide is derived by cleavage from the primary μ1 polypeptide[5]. The correspondence between genes and proteins is shown in Table 1.

Morphology. Reovirions are icosahedral virions comprising a double capsid(s), which consists of a core containing the 10 genome dsRNA segments and a closely applied inner protein shell surrounded by an outer protein layer. The polypeptides in the core include λ1–λ3, μ1, μ2 and σ2 (refs 5, 8). The core contains the viral transcriptase and replicase[5]. The outer capsid shell consists of three polypeptides σ1, μ1C and σ3 (Fig. 1). Protein λ2 is exposed on the outside of the virus, and is thought to represent the core spike[10]. It is closely associated with σ1, the viral haemagglutinin (HA)[10]. The two major outer capsid polypeptides are μ1C and σ3; these are complexed to each other and are found in a 1:2 ratio in the virion[5].

Role of outer capsid in pathogenesis of reovirus infection

The pattern of virulence of the mammalian reoviruses is determined primarily by the three outer capsid polypeptides (μ1C, σ1 and σ3)[3], each of which has a unique role. Following entry into the gastrointestinal tract (the presumed natural portal of entry), the μ1C protein is digested by host cell proteases. It is this that induces, in part, the cellular immune response[11]. The σ1 protein (the viral haemagglutinin) interacts with receptors on the surface of immune and non-immune lymphocytes and neurones and in this way determines cell and tissue tropism[12–16].

Table 1 Correspondence between reovirus dsRNA genes and polypeptides

Gene segment	Polypeptide	Location in virus
L1	λ3	Core
L2	λ2	Core spike
L3	λ1	Core
M1	μ2	Core
M2	μ1/μ1C	Outer capsid
M3	μNS	Nonstructural
S1	σ1	Outer capsid
S2	σ2	Core
S3	σNS	Nonstructural
S4	σ3	Outer capsid

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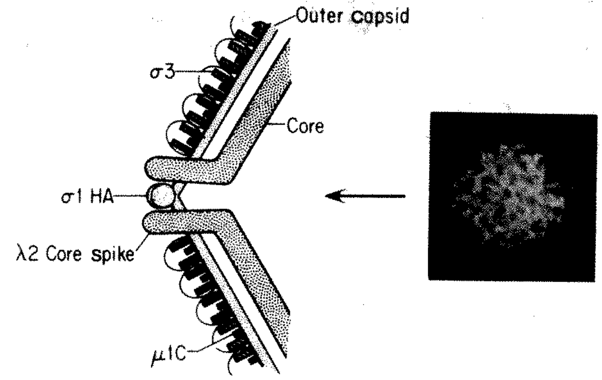

Fig. 1 Schematic diagram of the outer capsid of mammalian reovirus serotype 1, 2, 3. On the right is a negatively stained electron micrograph of an intact virion. On the left is a tentative schematic drawing showing how the three outer capsid proteins ($\sigma 1$, $\sigma 3$ and $\mu 1C$) and the core protein ($\lambda 2$) are organized on the outside of the virion: $\sigma 1$ is located at the vertices of the icosahedron close to $\lambda 2$, the core spike. $\mu 1C$ and $\sigma 3$ are associated with each other on the flat surface of the icosahedron.

It is the major antigen determining specific antiviral humoral or cellular immune responses. The $\sigma 3$ protein is responsible for inhibiting host cell RNA and protein synthesis and has a critical role in allowing the reovirus to initiate persistent infection of cultured cells[17,18].

Clearly, the three polypeptides of the outer capsid have different functions. Thus, altering any of them affects the behaviour of reoviruses in distinct ways (Table 2). The relevance of such an alteration to attenuation will now be reviewed in some detail for each protein.

Attenuation of mammalian reoviruses via the viral haemagglutinin ($\sigma 1$)

Allelic substitution. The major function of the haemagglutinin of reovirus in viral pathogenesis is determination of cell and tissue tropism. Intracerebral (i.c.) inoculation of newborn mice with type 3 reovirus leads to the development of an acute encephalitis that is uniformly fatal and associated with specific viral replication in neurones but not in ependymal cells[19,20]. In contrast, inoculation of type 1 reovirus causes a non-fatal acute ependymitis without involvement of neurones[21]. Localization of the different types of reovirus to different cells is determined in both cases by the $\sigma 1$ protein encoded by the *S1* gene[16,22]. Thus, substitution of the type 3 *S1* gene by alternative allelic forms of *S1* (from either type 1 or 2) can attenuate the highly virulent type 3 infection by targeting virus to less lethal sites[16]. It is important to emphasize that this kind of attenuation is associated with differences in specificity of the immune response of the host. The *S1* gene product is the antigen that evokes the major type-specific humoral and cellular immunological responses[13-15]. As immunity is type-specific, an infection with a reovirus containing a type 1 *S1* gene does not result in protective humoral or cellular immunity for reoviruses having different type 2 or 3 *S1* genes.

Selective mutations in the *S1* gene. To better define the functions of the viral haemagglutinin, we isolated several monoclonal antibodies directed against the $\sigma 1$ polypeptides[23]. As reovirus type 3 is 'neurovirulent', we concentrated largely on analysis of this serotype. We discovered several functionally distinct antigenic regions on the haemagglutinin, one of which is the major site recognized by neutralizing antibodies[23]. The same site is involved in the recognition of the reovirus type 3 haemagglutinin by cytolytic T lymphocytes (CTL), since the neutralizing monoclonal antibody blocks the interaction between such cells and reovirus[25].

As this site on $\sigma 1$ plays such a critical part in recognition of reovirus by the host immune system, we wished to determine whether it also has an important role in the interaction of reovirus with non-immune cells such as neurones. We reasoned that if the neutralization site of the viral haemagglutinin is involved in binding to receptors on the surface of neurones, variants resistant to neutralization by monoclonal antibodies might lose the capacity to bind to, or enter, neurones. When we isolated such variants by collecting those viral clones which grew in the presence of neutralizing monoclonal antibodies[24] and inoculated them into suckling mice, they were found to be either avirulent (lethal does for 50% of the population, $LD_{50} > 10$) or markedly reduced in virulence and were impaired in their ability to grow in the brains of newborn mice[24]. In contrast, the parental Dearing reovirus serotype 3, is highly neurovirulent ($LD_{50} \sim 10^1$) and grows to high titre in brain tissue. In fact, examination of brains of mice inoculated with these variants shows a tropism restricted to parts of the hippocampus (D. R. Spriggs, unpublished data). These observations indicate that when variants are selected for resistance to antibody directed at the major neutralization region of the viral haemagglutinin, viruses specifically attenuated for lethal central nervous system infection are generated. The most likely explanation for this type of attenuation is an altered interaction of the viral haemagglutinin with neurone receptors. In addition to being less neurovirulent, these variants show an altered interaction with cytolytic T lymphocytes[25]. Thus, either the identical site on the reovirus type 3 haemagglutinin protein is recognized by a neutralizing monoclonal antibody, cytolytic T lymphocytes and neurones, or the site(s) important for the last two interactions are close enough to that recognized by the antibody to be seriously affected by antibody binding.

Effect of antibodies against receptors that bind viral haemagglutinin. As suggested above, a decisive element in the successful interaction of the haemagglutinin with certain cells is a highly specific cell-associated receptor for the haemagglutinin. Because the receptor on lymphoid and neuronal cells is able to distinguish subtle differences in the structure of various haemagglutinins, it has been possible to develop a panel of xeno- and monoclonal antibodies to the viral receptor[26]. We reasoned that the antigen-binding site of antibodies that could interact with the neutralizing area of the haemagglutinin might resemble the binding site of the surface receptor on the neurone.

Table 2 Mutations attenuating reoviral virulence

Gene	Polypeptide	Function	Result of attenuating mutations	Ref.
S1	$\sigma 1$ (HA)	Binds to receptors: I nerve cells II lymphoid cells	Loss of neurovirulence Altered specificity in cytolytic T cell and humoral responses	24
M2	$\mu 1/\mu 1C$	I Growth at mucosal surfaces	Decreased capacity to grow at 1° site	28
		II Immune induction	Loss of immune stimulation of suppressor T lymphocytes	11
		III Growth in target tissue	Relative decrease in neurovirulence	26
S4	$\sigma 3$	Inhibition of RNA and protein synthesis	Persistent infection	16
			Interference	36
Multiple genes	Many proteins	Pleiotropic	Temperature-sensitive mutations have same tropism but reduced growth *in vitro* and *in vivo*	38

The fact that the same determinant recognizes the haemagglutinin suggests that the genetic basis of these binding structures on T lymphocytes or somatic cells (neurones) may involve a gene family similar to that which encodes immunoglobulin variable region products. Alternatively, we considered that limited conformational arrays necessary for HA-specific receptor construction might resemble the conformation of antigen-combining sites on antibodies having the same specificity[26].

BALB/c mice were inoculated with the BALB/c-derived monoclonal antibody (MC1) known to interact with the neutralizing region of the haemagglutinin. Spleen cells from these animals were fused to generate a second order panel of hybridomas, which were screened to isolate monoclonal antibody (MC2) potentially capable of interacting with the antigen-binding site of the first monoclonal antibody (John Noseworthy, unpublished). (Previously, we induced antibodies against anti-haemagglutinin by immunizing rabbits with anti-reovirus haemagglutinin antibodies[26].) These rabbit anti-anti-haemagglutinin antibodies have been used to mimic viral binding[27]. More recently, the MC2 antibodies have been found to limit binding of virus to appropriate targets (R. Kaufman, unpublished data). Thus, blocking viral receptors by MC2 prevents viral entry into susceptible cells and consequently the cells may be protected from infection.

The antibodies can also interact with lymphocytes in a manner analogous to the reovirus itself, as judged by the ability of such putative anti-receptor antibodies to activate subsets of T cells and to bind to a panel of cell lines which the virus binds[27]. We have observed, for example, that MC2 antibodies bind to cells which can interact with reovirus 3. This has been shown for various murine cell types and tumour cell lines of distinct H-2 types, as well as distinct heavy chain allotypes. The YAC (A strain, *H-2*^{a}) lymphoma line binds neither reovirus 3 nor MC2, but the R1.1 (C58, *H-2*^{k}) thymoma binds both reovirus and the MC2. Absorption of the MC2 with the R1.1 line also absorbs out the activity of MC2 binding to MC1, indicating that MC1-like viral receptors are present on the cell surface. This type of MC2 is not only of value in blocking binding of virus to cells but may be useful also *in vivo*, particularly against local surface infections. As the MC2 resembles a viral antigen, it might also be used to induce or augment immunity to viral diseases without exposure of the host to the virus. This strategy might be most advantageous in the development of an immunogenic 'vaccine', for protection against infection by more highly lethal viruses, for example, the agent responsible for rabies.

Another area of interest is the simple definition of an analogue of virus–receptor interactions by using two monoclonal antibodies, MC1 and MC2: MC1 resembles the receptor and MC2 mimics the virus. It is now possible to assess readily which of several tryptic peptides of the haemagglutinin is capable of inhibiting MC1–MC2 interaction. Those peptides which inhibit this interaction in a radioimmunoassay could probably generate immunity to the most relevant part of the viruses when administered *in vivo*. We predict that this principle (see Fig. 2) should also be applicable to other viruses and may allow the rapid screening and development of new vaccines (synthetic peptides, protein fragments, synthetic compounds). An alternative approach uses reovirus fixed to plates, and radiolabelled MC1 antibody. Tryptic or synthetic peptides of haemagglutinin can then be tested for their ability to inhibit the radiolabelled MC1 binding to the immobilized reovirus. Those peptides capable of inhibiting the interaction would be candidates for immunogens in vaccines. Again, the principle should also extend to other viral systems.

Attenuation via the μ1C protein

Effect of route of inoculation on virulence. The Dearing strain of reovirus serotype 3 is highly lethal when inoculated i.c. into suckling mice, a typical LD$_{50}$ being ~1 log$_{10}$ (ref. 16). However, when this strain is given orally, even in doses of 10^7 plaque-forming units (PFU) of purified virus, it is avirulent[28]. This quantity of type 3 reovirus fails to grow in intestinal tissue after it is deposited into the stomach of newborn mice and is inactivated when mixed with intestinal homogenates. In contrast, reovirus type 1 (Lang) strain inoculated by the same route multiplies in the intestine, then spreads to the brain where it produces a benign ependymitis similar to that which develops after intracerebal injection. Another difference between the two viruses is that the infectivity of the type 1 is unchanged or slightly enhanced by treatment with pancreatic protease chymotrypsin while that of the type 3 (Dearing) is dramatically decreased[28]. A genetic analysis using recombinant viruses generated between types 3 and 1 revealed that the ability to grow in intestinal tissue and the response to pancreatic proteases are properties of the M2 gene and hence the μ1/μ1C proteins[28], but is uncertain whether the two are causally linked.

Effect of variations of M2 on neurovirulence. In addition to determining growth in intestinal tissue, the *M2* gene plays a part in controlling the relative degree of neurovirulence of a natural isolate of serotype 3 (ref. 29). A large number of type 3 isolates were screened to ascertain their relative neurovirulence after i.c. inoculation. One strain retained the neurotropism of the type 3 (that is, infection of neurones but not ependyma) but was markedly less virulent (LD$_{50}$ of ~$10^{4.5}$ compared with type 3 Dearing LD$_{50}$ of ~10^1). Genetic analysis revealed that the attenuation in neurovirulence in this isolate was also related to its *M2* gene[29].

The results of these studies indicate that the nature of the *M2* gene (and μ1C polypeptide) is important in determining virulence after inoculation by the natural oral route and by the experimental intracerebral route. This effect seems to be mediated by how well reovirus grows either at the portal of entry or in the target central nervous system tissue. Thus, while tissue tropism is not affected by the *M2* genes, the ability of reovirus to grow and produce a lethal lytic infection is. The manipulation of the *M2* gene thus offers the possibility of attenuating reoviruses by reducing virus yield.

Induction of immunity. As indicated previously, the μ1C protein is normally sensitive to various proteases present in the

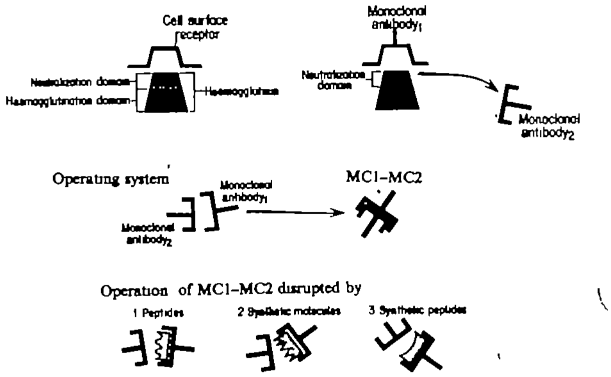

Fig. 2 Definition of an operational analogue of virus–cell receptor interactions. In the reovirus system, the neutralization domain of haemagglutinin interacts with cell surface receptors and the antigen-binding site of the monoclonal antibody (MC1) directed at that domain. A second monoclonal antibody (MC2) made against the antigen-binding site of the first interacts with the cell surface receptor for the neutralization domain, and also interacts with MC1. The MC2–MC1 interaction appears to be the analogue of the virus–cell receptor interaction and constitutes the operating system for studying proteins which can be used as potential immunogens. Only those proteins (or synthetic molecules) that interfere with MC1–MC2 binding are likely to be useful vaccines. In cases where the virus is not dangerous and easily purified, an alternative approach is possible: the virus is immobilized on polystyrene plates, and MC1 antibodies (directed against the domain of the virus that interacts with cell receptors) are radiolabelled and used to bind the immobilized virus. Any peptide or synthetic molecule which limits the binding of the radiolabelled MC1 to the virus is potentially useful as a vaccine. These systems could apply also to bacteria, fungi or parasites[34,35].

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intestine[28]. However, oral administration of a virus having a mutant μ1C protein, resistant to intestinal enzymes, stimulated suppressor T cells (T_s) which abrogated the cellular immune response to the virus[11]. Thus, the μ1C polypeptide encoded by the *M2* gene plays a decisive part in the overall pattern of the immunological response when reovirus enters the host by the natural route. One possible explanation of these results is that a virus carrying μ1C that is resistant to enzymatic cleavage is processed in a manner which stimulates T_s activity. Evidence for special antigen-presenting cells in the generation of T_s has, in fact, recently been demonstrated (Adam Lowy, Jeffrey Drebin and M.I.G., unpublished results).

Attenuation via the σ3 protein

Effect of high passage on virus properties. Viral vaccines are often prepared by multiple passage of stock virus until attenuation occurs. The critical genetic lesions of most vaccine strains have not been identified. Many viral vaccines are partially temperature-sensitive (ts) suggesting that they may contain multiple mutations[30–32]. As the segmented genome of the reoviruses has allowed us to determine the locus of ts lesions, we investigated the nature of the ts and other lesions produced in reovirus types 1, 2 and 3 after serial, lytic passage[33]. Although scoring for every possible mutation was not feasible, several mutations were detected in these stocks, including ts mutants, deletions, cold-sensitive and non-ts interfering lesions. At several stages of passage, most of the ts and non-ts interfering mutants appeared in the tsG group, which has been found on the S4 gene encoding σ3 protein[34]. Thus, after repeated passage of reovirus in tissue culture performed in a manner analogous to generation of attenuated strains, the major mutations appear in the *S4* gene.

Defective interfering particles. Defective interfering (DI) particles are virus particles which lack a portion of the viral genome, contain normal structural proteins, require helper virus to replicate and interfere specifically with replication of homologous standard helper[35]. Reovirus DI particles are generated after serial passage of ts^+ or ts mutants in cell culture[36–38]. Reovirus DI particles often appear after several passages with ts^+ viral stocks, but may be detected earlier in stocks of ts mutants when passage is performed repeatedly. At high multiplicity of infection (MOI), the L1 dsRNA genome segment is most often lost, but deletion mutants lacking segments *L3* and *M1* have also been found. These replicate only in the presence of standard virus, and interfere with the growth of infectious reovirus.

DI viruses are highly attenuated. Because the precise mechanisms involved are unknown, the genetic interactions between reovirus DI and infectious virus were examined[39]; the results indicate that ts^+ recombinants form after crosses between reovirus DI particles and ts mutants or ts^+. We then asked whether DI particles contain other mutations, in addition to the deletion mutation. It was clear that ts and small plaque–low yield mutations are rescued from DI particles; both of these mutations were located on the *S4* gene segment. Indeed, some of the ts and attenuated viruses constructed from these crosses between DI particles and ts were able to interfere with the growth of wild-type reoviruses. Thus, like the repeated passage non-DI stocks, the more defective DI particle stocks were also highly enriched for viruses containing *S4* mutants.

Virus isolates from persistent infection. DI viruses are critical for the establishment of persistent infection in cell cultures[40]. By infecting L cells with DI stocks, a noncytocidal persistent infection can be established. To determine which gene(s) derived from the DI stock play(s) a critical part in the establishment of persistent infection, cells were co-infected with DI stocks and ts^+ virus[17]. If mutation in a particular gene is essential for the establishment of persistent infection, it should always be derived from the defective virus. Studies designed to examine this hypothesis indicated that the *S4* gene segment was always derived from the defective virus and was critical for attenuation in that it specifically produced non-lytic virus–cell interactions. Interestingly, a major role of the σ3 protein in the cell during lytic infection is to inhibit RNA and protein synthesis[18]. Mutant σ3 derived from persistently infected cells, has lost the capacity to do this. Thus, mutations in *S4* are attenuating because they are altered in their ability to lyse cells and inhibit synthesis of cellular protein and RNA. The *in vivo* significance of *S4* mutations has not yet been evaluated.

Temperature-sensitive mutations

That reovirus type 3 Dearing produces a lethal encephalitis after intracerebral inoculation has been discussed above. We have also pointed out that attenuation due to specific lesions in individual viral genes is often associated with viruses capable of full replication is cell cultures. In contrast to this specific type of attenuation, ts mutants whose lesions may be located in one of several viral genes are highly attenuated compared with parental wild-type virus[41]. Temperature-sensitive mutants are unable to replicate fully at relatively high temperatures such as the body temperature of the infected host. The extent of the attenuation associated with infection with ts viruses depends in part on the nature of the lesion. However, we now know the location of the lesions used in all our earlier studies and it is clear that attenuation of lethal encephalitis occurs with lesions in the reovirus core as well as with lesions in outer capsid proteins[42]. The attenuation is characterized by an intermediate level of destruction of neurones (that is, 'attenuation') that may be due to a virtually complete block in viral replication (ts C) or a block in replication at more intermediate levels (ts B)[41]. In no case have we found evidence of altered tropism with the reovirus ts mutants, although studies using vesicular stomatis indicate that this may occur[43].

Conclusions

For a virus or other microbial agent to produce infection in eukaryotic host it must enter through a major portal, spread (if it produces systemic disease) and ultimately find a distinct cell and tissue site. The host responds to the pathogen both at the portal of entry as well as systemically. Several stages of pathogenicity are common to all types of infectious agents, from the simplest viruses to complex parasites. Because viruses are the smallest and simplest of the infectious agents, they offer the possibility of establishing general principles that explain molecular mechanisms of microbial pathogenecity.

Several conclusions regarding features of attenuation of viral virulence and immunization emerge from this review. First, one viral component, the haemagglutinin, is responsible for determining cell and tissue tropism and is a major antigen for the humoral and cellular immune responses. Selection of viruses that are variant in critical regions of the haemagglutinin leads to attenuation. In addition, presumed anti-receptor antibodies generated against monoclonal antibodies directed against the binding site of the haemagglutinin, potentially can be used both to stimulate an immune response against the virus and possibly to block binding of virus at the level of the cell receptor. Such systems as those which we have defined by MC1 and MC2 monoclonal antibodies are analogues of the virus–cell receptor system and should permit the rapid *in vitro* screening of peptides, synthetic peptides and even synthetic compounds for use as vaccines.

Second, a viral component of the outer capsid, the μ1C protein, has a critical role in determining the ability of reovirus to replicate at the primary site in the gastrointestinal tract and the ultimate nature of the immune response. Thus, this component provides a second site on the surface of reovirus particles that can be altered, resulting in a reduced capacity of the virus to grow and interact with the host at portals of entry. Third, the σ3 outer capsid polypeptide, the component responsible for inhibiting synthesis of cellular RNA and protein, is the site of frequent mutations that decrease the capacity of reovirus to lyse cells. Such mutations often appear in viral stocks after they have been repeatedly passaged, and are present at high frequency in defective interfering particles. The presence of such S4 mutations is associated with attenuation in conditions where defective viruses are present.

Are other microorganisms similar to the mammalian reoviruses in the strategies they use in interacting with eukaryotic hosts during infection, and in the ways that the reoviruses can be attenuated? Although our conclusions are based largely on the results of studies using the reoviruses, it is possible that the lessons of the reoviruses will apply to other microorganisms. That different facets of viral virulence are properties of multiple viral genes has also been noted for the influenza viruses[4,44,45]. Despite the fact that virulence is multigenic and that multiple genes are involved in infection, individual viral genes are probably responsible for different facets of the process. For example, studies with a number of viruses including poliovirus[46], coxsackie virus[47], adenovirus[48], paramyxoviruses[49], and influenza virus[50], have emphasized that generally only one of the viral polypeptides is responsible for interacting with host receptors. While most of these investigations have described experiments using cell cultures rather than animal models, it is likely that similar results will be true *in vivo*. Note that, in most cases, it is the viral haemagglutinin that is the protein responsible for receptor interactions. The detailed mechanisms involving subsequent stages of viral infection involve different proteins and generally are not well understood. For example, influenza virus contains a second outer capsid protein, the neuraminidase, thought to have a role in allowing the virus to come into close contact with the target cell and possibly to aid release of virus from infected cells[51]. In addition, several viruses contain a fusion protein that is thought to participate in initiation of infection and expression of infectivity[52]. Clearly, discrete viral proteins, in addition to the haemagglutinin, are responsible for additional stages of infection.

With regard to aspects of the responses of hosts to viruses, an oligoclonal response as typified by the anti-haemagglutinin antibodies for reovirus can also be seen in the immune response to the influenza viral haemagglutinin. Studies by Liu *et al.*[53], for example, have clearly identified a variety of idiotypes against overlapping antigenic determinants on B/Lee haemagglutinin. They found both private as well as shared idiotypes and, in some cases, they detected totally cross-reactive idiotypes on monoclonal antibodies generated against a particular antigenic site. Recently, the effect of anti-idiotypic or anti-receptor reagents against other microorganisms have also been noted[54,55]. Such reagents may help to elucidate the binding structure for a variety of microbial agents and define new strategies of therapy for modulating microbial infection.

Thus, the increased understanding of the mechanisms of microbial pathogenesis that is based on model systems should provide important new approaches for the prevention and treatment of infectious diseases caused by diverse microorganisms.

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ARTICLES

Observations of reverse polarity flux transfer events at the Earth's dayside magnetopause

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Observations of flux transfer events (FTEs) at the Earth's dayside magnetopause are presented which have plasma and magnetic field signatures reversed in sign from those previously reported. These FTEs are interpreted in terms of localized and transitory reconnection with the spacecraft located south of the reconnection site. They are associated with a previously reported interval of quasi-steady reconnection which is similarly located, indicating that a close physical connection exists between the two processes.

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THE occurrence of large-scale plasma convection within the Earth's magnetosphere, driven by the external flow of the solar wind, has long been recognized as being of fundamental significance in determining the properties, structure and dynamics of magnetospheric plasma populations. There has, however, been much controversy concerning the nature of the physical processes which couple the magnetosphere to the external shocked solar wind (magnetosheath) and which initiate the convection cycle by transporting plasma and magnetic flux from the dayside boundary into the geomagnetic tail. The two main possibilities which have been discussed have been a 'viscous-like' interaction associated with wave–particle interactions and plasma diffusion at the magnetopause boundary[1,2], and magnetic reconnection, in which direct magnetic interconnection occurs between the solar wind and the magnetospheric flux tubes, resulting in these 'open' tubes being swept downstream into the tail by the magnetosheath flow[3]. These two types of processes may, however, coexist, and the determination of their relative importance in driving magnetospheric convection is a central problem in magnetospheric physics. Indirect evidence has supported the importance of reconnection but direct confirmatory observations have been lacking, due to the lack of data of sufficiently high time resolution from the dayside boundary regions.

Recently, high resolution plasma and magnetic field measurements made by the ISEE 1 and 2 spacecraft have become available, and have provided the first definitive observations of magnetic reconnection at the dayside boundary. These observations have shown that the reconnection can occur either as a large-scale, quasi-steady process enduring on time scales of at least a few tens of minutes[4–6] as originally envisaged by Dungey[3], or as a transient process, localized in both temporal and spatial extent. The latter form, termed the 'flux transfer event' (FTE), was first recognized by Russell and Elphic[7,8] from its characteristic magnetic signature in ISEE magnetosheath data, although apparently related field fluctuations inside the magnetopause boundary had previously been observed in low resolution HEOS 2 data, and described as possible 'flux erosion' events by Haerendel *et al.*[9]. The interpretation of such field phenomena in terms of localized reconnection has been supported by particle observations indicating that a mixture of magnetospheric and magnetosheath plasma occurs in FTE flux tubes both inside and outside the magnetopause[10–16].

Most of the flux transfer events discussed recently were those observed early in the ISEE 1–2 mission in November 1977 when the spacecraft encountered the magnetopause region on the dawn side of the Earth and at relatively high northern magnetic latitudes (20–40°). All of these encounters have been interpreted in terms of a spacecraft location northward of the reconnection region, such that the open FTE flux tubes which were observed were connected to the Northern Hemisphere of the Earth. The signatures associated with the passage of such a tube across the spacecraft may be readily envisaged with the aid of Fig. 1, following Russell and Elphic[7,8]. This diagram represents a view of the dayside magnetopause as observed from the magnetosheath, and shows the two 'ends' of a magnetosheath flux tube which has become connected across the magnetopause into the northerly directed (vertical) field of the Earth on the other side of the boundary as a result of a localized reconnection event. Due to magnetic tension, the reconnected flux tubes will contract along the magnetopause in the directions indicated by the broad arrows relative to the overlying magnetosheath plasma and field, causing perturbations in the latter as they do so, as illustrated. If we then consider the magnetic perturbations which should be observed by a spacecraft as the northern open tube passes across it, moving roughly in the direction of the broad arrow, then it is clear that the field will point outwards, away from the magnetopause as the open tube approaches, and inwards, towards the Earth as it recedes, while within the tube strong deflections of the field direction towards that of the Earth's field are expected. The overall plasma flow within the open tube should be deflected both to the north and to the west relative to the ambient magnetosheath flow due to the magnetic forces (the latter direction clearly depending on the sign of the east–west magnetosheath field component). This basic picture is quite consistent with previous FTE observations presented in refs 7, 8, 13 and 17. In particular all have shown the positive to negative signature of field perturbations directed along the outward normal to the magnetopause, and for those cases where a significant flow perturbation is observed it is directed northwards. Not all the FTEs show clear and consistent northward velocity perturbations however, making their identification as Northern Hemisphere open tubes rather less certain in these cases[16].

If we now consider the southerly tube in Fig. 1, again assumed moving roughly in the direction of its broad arrow, then the perturbations expected in both the magnetopause normal field component and in the plasma flow are reversed in sign relative to the northern tube, while deflections of the reconnected flux tube in the plane of the magnetopause should remain the same, that is northward towards the direction of the Earth's field[16]. We present here the first observations corresponding to this situation as observed by the ISEE spacecraft.

Observations

Our data were obtained by the ISEE 1 spacecraft during an inbound crossing of the magnetopause region on 9 August 1978 which occurred at a radial distance of $\sim 15 R_E$ and at a local time and magnetic latitude of ~1650 h and ~2° respectively. The spacecraft was thus located in the dusk local time sector and close to the magnetic equator. Plasma and magnetic field data for a 70-min interval spanning the magnetopause region are shown in Fig. 2. The plasma data were obtained by the Los Alamos/MPI fast plasma analyser and were taken from ref. 5, in which further details of the experiment may be found. Figure 2 shows the plasma density N_p (cm^{-3}) and flow speed in the equatorial plane V_p (km s^{-1}), both obtained at 12 s resolution, together with the GSM *z* (northward) component of the plasma velocity V_z (km s^{-1}) obtained at 48-s resolution. The magnetic field data were obtained from the UCLA fluxgate mag-

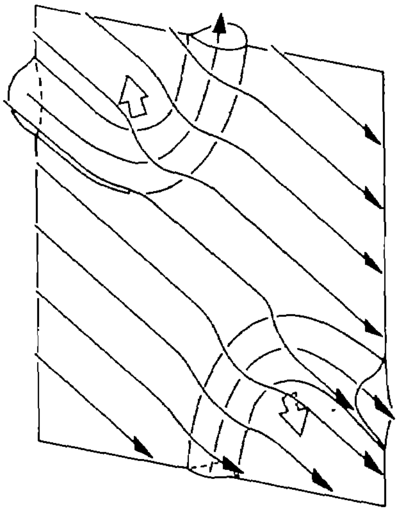

Fig. 1 Sketch of northern and southern 'ends' of open FTE flux tubes shown shortly after reconnection at the dayside magnetopause. The magnetic tension of the open tubes will result in their contraction along the magnetopause relative to overlying magnetosheath plasma and field in the directions indicated by the broad arrows. Consequently the open magnetosheath tubes will tend to tilt towards the direction of the Earth's field (northward) as shown, while the open magnetospheric (boundary layer) tubes will tend to tilt towards the magnetosheath field direction, both north and south of the reconnection site. Perturbations will also occur as shown in the overlying magnetosheath field, particularly in the field component normal to the magnetopause. If the net motion of the open tubes across a spacecraft is roughly as indicated by the broad arrows then the normal field perturbations will be outward followed by inward for the northern tube, and inward followed by outward for the southern tube.

netometer on ISEE 1 (ref. 18), and 12-s field averages plotted every 4 s are shown in boundary normal coordinates. In the latter system $\hat{N}$ is the estimated outward normal to the magnetopause at the spacecraft location and points duskward and sunward nearly in the equatorial plane. $\hat{L}$ is perpendicular to $\hat{N}$ and such that the L-N plane contains the GSM $\hat{z}$ axis; it is directed northward along the magnetopause. $\hat{M}$ completes the orthogonal system and points sunward and dawnward along the magnetopause. In Fig. 2 $\hat{N}$ was determined by assuming that to a sufficient approximation the magnetopause can be treated as a tangential discontinuity, in which the normal magnetic field component threading the current sheet is taken to be zero. The $\hat{N}$ direction is then given by the cross product of representative field vectors on either side of the boundary, from which its GSM components were found to be (0.753, 0.644, 0.139). This procedure does not really conflict with the possibility that the magnetopause is magnetically open during this traversal as the strength of the normal field is generally expected to be around an order of magnitude less than that of the tangential components. Note also that the above normal is in reasonable agreement with the Fairfield[19] model magnetopause normal for the spacecraft location and also does not differ significantly for our purposes from the minimum variance normal employed by Sonnerup *et al.*[5]. The angle between the Fairfield normal and Sonnerup *et al.* normal and that used here is ~14° in both cases.

The basic features of the 9 August 1978 magnetopause passage may be readily discerned from the plasma and field parameters displayed in Fig. 2. Initially the spacecraft is located in the magnetosheath as can be seen from the high plasma density and bulk speed, and from the southward and duskward (tailward) field directions (that is, B_L and B_M are both negative). There then follows an interval from 1934 to 1953 UT which is interpreted by Sonnerup *et al.*[5] as a partial penetration of the magnetopause/boundary layer region. The magnetosheath is then re-entered, until at 2011 UT a final crossing of the magnetopause occurs after which the magnetic field takes on its northward and sunward (dawnward) magnetospheric direction (that is B_L and B_M are both positive). Inside the magnetosphere after the final magnetopause crossing several pulses of flowing boundary layer plasma also occur.

From a detailed examination of the plasma, energetic particle and magnetic field data Sonnerup *et al.*[5] were able to deduce that quasi-steady reconnection was taking place in the vicinity of the spacecraft during this magnetopause passage. In particular it was inferred that the magnetopause/boundary layer region observed between 1934 and 1953 UT was located on open magnetic field lines southward of the reconnection region. In Fig. 2 this is indicated by the substantial increases of plasma flow speed observed during this interval, and the southward deflection of its direction. These changes in the plasma flow were shown to be quantitatively consistent with the requirements of tangential stress balance across an open magnetopause with B_N positive (that is, on open tubes connected to the Southern Hemisphere of the Earth). The tangential magnetic field during the 1934 to 1953 UT interval is seen to be intermediate in direction between magnetosheath and final magnetospheric values, that is it is directed predominantly northward and tailward. In the terminology of Hones *et al.*[20] this corresponds to an example of 'reverse draping' of the boundary layer field (that is tailward field tilts north of the Equator) on the dusk side of the magnetosphere. In this case, however, the direction of the boundary layer field tilt clearly relates to the direction of the field in the magnetosheath (where B_M is negative) and is just that expected for open tubes being pulled tailward from a reconnection site north of the spacecraft[21]. This observation may help explain the unusually lengthy nature of this apparent 'magnetopause' encounter.

The main feature of the data to which we wish to draw attention here, however, is the occurrence of two prominent FTEs which occur on either side of the quasi-steady open boundary layer region described above. These can be seen in Fig. 2 at ~1921 and ~2004 UT and they display all the classic FTE properties described by Russell and Elphic[7,8] and Paschmann *et al.*[13] (their category C), except for the negative to positive polarity of the B_N signature and the small but clear southward deflections of the magnetosheath plasma flow. These properties are the reverse from those reported for such events in the literature so far. Properties which remain the same as those previously reported include the following:

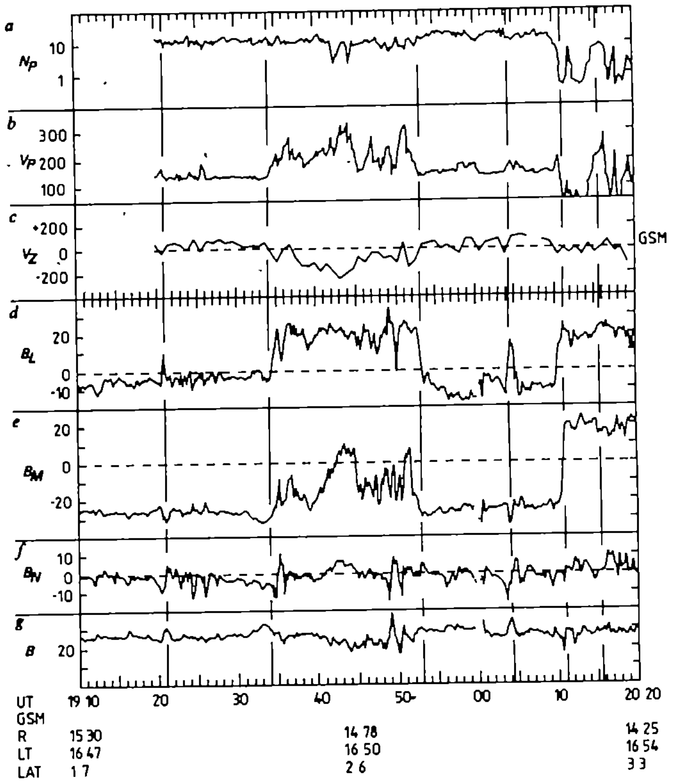

Fig. 2 Plasma and magnetic field data spanning the magnetopause boundary region obtained by ISEE 1 on 9 August 1978. *a–c* Plasma data obtained by the Los Alamos/MPI fast plasma analyser and published by Sonnerup *et al.*[5]. *a* and *b* show the plasma density N_p (cm^{-3}) and equatorial plane bulk speed V_p(km s^{-1}) respectively, both obtained at 12 s resolution while *c* shows the GSM *z* (northward) component of the plasma velocity V_z (km s^{-1}) obtained at 48 s resolution. The magnetic field data in *d–g* are 12 s averages plotted every 4 s and shown in boundary normal coordinates. A ~1 min gap in the ISEE 1 magnetic field data centred on ~2000 UT has been filled by ISEE 2 data in the figure, as indicated by the small dotted interval. Note that the ISEE 1 and 2 field data are essentially identical throughout this traversal, due to a small spacecraft separation. The separation vector in *L*, *M*, *N*, coordinates from ISEE 1 to ISEE 2 was ~45, 80, 65 km. The flux transfer events can be seen at ~1921 and ~2004 UT.

(1) The direction of the tangential field is deflected strongly towards that of the Earth's field as indicated by the changes in both B_L and B_M components (the latter satisfying $\Delta B_M/B_M > 0$).

(2) Both the magnetic pressure and the plasma pressure (see ref. 5) are considerably enhanced during the events.

(3) The events occur for southward directed magnetosheath fields ($B_L < 0$).

These properties are clearly those anticipated above for the propagation of localized reconnected FTE flux tubes across the spacecraft which are located south of the reconnection line and connected to the Southern Hemisphere of the Earth.

Note that the negative to positive B_N perturbations in these FTEs extend well outside the interval in which the open tube itself is observed, the latter interval being defined by the sharp northward tilt of the field in the plane of the magnetopause. These extended B_N perturbations result from the distortion of the magnetosheath field overlying the open tube as depicted in Fig. 1, and tend to maximize near the open tube boundaries as expected. Figure 2, however, shows that B_N perturbations are also present within the open tube itself as previously noted for 'normal' polaritiy FTEs[13,16]. The appearance of these B_N fields suggests that the open field lines are twisted about the axis of the FTE flux tube, associated with the presence of a distributed field aligned current (FAC) which is flowing along it. The direction of current flow in the present case is deduced to be parallel to the magnetic field from magnetosphere to magnetosheath, compatible with the direction of low-altitude 'region 1' currents in the dusk sector. Similar conclusions apply to previously published 'normal' polarity FTEs in the dawn sector which again show current flow parallel to the magnetic field, but now directed from magnetosheath to magnetosphere as the tubes are connected to the Northern Hemisphere of the Earth[16]. In all these cases the sense of the B_N perturbations observed inside the open tube is the same as those occurring outside the open tube which arise from the distortion of the overlying magnetosheath field, thus resulting in a simple overall bipolar B_N signature. In some circumstances the senses of the B_N perturbations arising from these two sources may differ, so that more complex FTE B_N behaviour may then be anticipated.

Note that in principle a 'reverse' negative to positive B_N FTE signature arising from distortion of the overlying magnetosheath field could also be produced at dusk by the northern tube shown in Fig. 1. This could occur when the tailward-directed magnetosheath flow becomes sufficiently rapid near the magnetospheric flanks that such an open tube can be carried predominantly tailward by the flow against the direction indicated by the magnetic tension (the broad arrow in Fig. 1). This is unlikely to be the case at the ISEE location in Fig. 2 as the magnetosheath flow speed and the Alfvén speed are comparable in magnitude (~150 km s^{-1}). One could then expect to observe a reduction, if not a reversal in the equatorial plane flow, together with a northward deflection in its direction. The observed variations of the flow do not show such behaviour, but rather are in the opposite direction. We therefore feel that the observations indicate that the 'reverse polarity' FTE events which occur at ~1921 and ~2004 UT in Fig. 2 are connected to the Southern Hemisphere. This conclusion has been confirmed by examination of energetic (>45 keV) ion data, which show that both FTEs are associated with enhanced fluxes of ions, inferred to be of magnetospheric origin, streaming parallel to the field from the magnetosphere into the magnetosheath. (P. W. Daly, personal communication).

Finally note that magnetic field and plasma fluctuations which seem to be closely related to the above FTEs can be seen in Fig. 2 in the interval ~1922–1928 UT following the first clear FTE, as the spacecraft enters the magnetopause region at ~1935 UT, within the latter region at ~1949 UT and in the second magnetosheath interval at ~2000 UT. Other events were also observed before and after the interval displayed. In addition, the large pulse of accelerated boundary layer plasma observed at ~2015 UT after the final magnetopause crossing appears to be associated with a 'reverse polarity' negative to positive perturbation in B_N, together with a ~20° tailward tilt of the boundary layer field lines towards the direction of the magnetosheath field relative to adjacent magnetospheric field directions. This behaviour of the boundary layer field corresponds to that expected for a magnetospheric open FTE flux tube reconnected north of the spacecraft, although the large general level of magnetic fluctuations occurring in the magnetospheric boundary in Fig. 2 makes this identification uncertain. The interpretation of pulse-like boundary layer encounters such as this in terms of magnetospheric FTEs has been dicussed previously in refs 12, 13, 16 and 22.

Discussion and conclusions

From an analysis of ISEE 1 plasma and magnetic field data Sonnerup *et al.*[5] have shown that quasi-steady reconnection was occurring at the dusk magnetopause during an inbound crossing of the boundary region on 9 August 1978. They showed that the spacecraft was located south of the reconnection region at this time, the only such example found in the 11 reconnection events studied by the above authors. This example was also the most southerly located of these events, being only ~2° N of the magnetic equator compared with ~20° to ~40° N typical of the other encounters. We have shown that both before and after the ISEE encounter with the quasi-steady open boundary layer region studied by Sonnerup *et al.*[5] impulsive reconnection was occurring at the magnetopause as evidenced by the observation of flux transfer events in the plasma and magnetic field data. The signatures of these FTEs differ from those previously reported, however, in that the polarity of the B_N field perturbations is from negative to positive rather than vice versa. This, taken together with the small but clear southward deflections of the plasma flow observed during the events and the energetic ions flowing parallel to the field strongly indicates that these open FTE flux tubes were, like the quasi-steady open boundary layer flux tubes, located south of the reconnection region and hence connected to the Southern Hemisphere of the Earth at this time. As regards the actual position of the reconnection region little can be said at present other than that essentially all the available evidence points towards a roughly equatorial location, possibly tilting north–south away from the subsolar region in response to the IMF B_y component (see for example, refs 23–26) to accommodate results such as the above.

It is significant that FTEs with the above characteristics indicative of connection to the Southern Hemisphere should appear in close association with observations of a quasi-steady open boundary layer region which is inferred to be similarly connected. This association strongly suggests that a close link exists between the two observed reconnection phenomena. At least one other quasi-steady reconnection event is also known to be associated with the appearance of a magnetosheath FTE during the crossing of the boundary region[5,13]. This case was observed on 11 September 1979 near local noon at ~22° north magnetic latitude, and is consistent with the reconnection processes being located south of the spacecraft at that time.

It will be interesting to examine other quasi-steady reconnection events for the presence of associated FTEs. The observations reported here indicate that during the spacecraft penetration of the boundary region the reconnection which was occurring evolved from being unsteady to steady and back again in the local time sector sampled. Whether this represents a basically temporal sequence or rather a spatial pattern which moves into and then out of the spacecraft vicinity remains unclear.

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Environmental radioactivity in Cumbria

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The distribution of radioactivity discharged from Sellafield in Cumbria (north-west England) has been measured against a background of nuclear weapon fallout, with the object of determining the pathways and transfer mechanisms between various environmental media.

THE natural radioactivity in the environment arises primarily from uranium and thorium, including the series of decay products, and from potassium. Nowadays, the naturally occurring radioactivity is overlain by artificial radioactivity deposited with the debris from more than 30 yr of nuclear weapon testing. Most of this material, having been injected into the stratosphere, is deposited on a global scale according to a recognized latitudinal pattern[1].

Possible emissions by the nuclear power industry, from reactors or processing plants, will have constituents similar to the radioactivity of the nuclear weapon fallout, namely a mixture of the transuranic elements, fission products and nuclides in gaseous form such as tritium and ^{14}C. To distinguish the emissions from the fallout it would be necessary to measure the former against baselines set by the latter. As an indication of the scale of the effect of nuclear weapon tests, about 500 Ci of Pu had been deposited in the United Kingdom by 1980[2]: the current contribution of the external radiation from deposited weapon fallout (mainly ^{137}Cs) amounts to about 3% of the natural background[2]. Radionuclides from weapon tests have been measured continuously for nearly three decades: the cumulative deposition has been assessed directly in a more recent programme of soil sampling throughout Britain. Figure 1 shows the deposition countrywide of ^{137}Cs from nuclear weapon fallout up to 1977 plotted in contour form[3].

To investigate the environment of the Sellafield (Windscale) establishment in Cumbria (north-west England), it is necessary first to measure the distribution of radioactivity in various environmental media. Then it would be possible to study the mechanisms of transfer between these media, for example, between sea and land, within soil and between soil and plants. Because the amounts of radioactivity are so low the sensitivity of measurement of the radionuclides must be much greater than that required for monitoring according to the normal radiological limits. Artificial radioactivity in Cumbria will arise from: fallout from nuclear weapon testing; discharge to air from the Sellafield works; and discharge to sea from the Sellafield pipelines.

Table 1 Cumulative deposition of ^{137}Cs and Pu in Cumbria grassland (1978)

	Locality	Average annual rain (mm)	Deposition (pCi cm^{-2}) ^{137}Cs (0–15 cm)	^{137}Cs (0–30 cm)	$^{239+240}$Pu (0–15 cm)	^{238}Pu (0–15 cm)	Ratio ^{137}Cs/$^{239+240}$Pu (0–15 cm)
1	Biggar	940	8.0	15.3	0.28	0.019	29
2	Rampside	1,026	11.2	16.6	0.16	0.009	70
3	Lindal-in-Furness	1,195	12.0	16.6	0.18	0.011	67
4	Flookburgh	1,120	13.1	17.7	0.38	0.030	35
5	Little Annaside	1,094	7.4	9.8	0.30	0.031	25
6	Beckside	1,399	32.1	38.8	0.33	0.006	97
7	Grizebeck	1,650	12.2	19.0	0.24	0.012	51
8	Abbots Reading	1,470	10.4	13.5	0.16	0.006	65
9	Eskmeals	1,094	23.4	33.8	0.41	0.036	57
10	Stainton	1,259	14.9	16.9	0.30	0.010	50
11	Seathwaite	2,020	19.4	20.2	0.30	0.015	65
12	Hawkshead	1,815	13.1	19.5	0.25	0.014	52
13	Calder Bridge	1,084	36.9	41.4	1.12	0.034	33
14	Wasdale	1,570	15.4	22.2	0.83	0.020	19
15	Great Langdale	3,189	13.3	17.9	0.34	0.010	39
16	Ambleside	2,131	17.4	24.7	0.29	0.012	60
17	Kinniside	1,795	12.9	18.4	0.28	0.011	46
18	Ennerdale	2,344	21.7	25.7	0.80	0.020	27
19	Borrowdale	2,469	25.4	33.3	0.41	0.019	62
20	Patterdale	2,431	12.2	14.7	0.34	0.010	36
21	Ullock	1,340	11.5	15.3	0.22	0.004	52
22	Little Broughton	1,800	10.9	13.5	0.24	0.006	45
23	Lonscale	1,820	10.0	16.2	0.23	0.008	44
24	Threlkeld	1,288	7.0	8.5	0.13	0.003	54
25	Maryport	958	9.6	11.2	0.41	0.019	23
26	Cockermouth	1,279	9.7	14.8	0.38	0.015	26
27	Uldale	1,561	10.1	14.4	0.17	0.009	59
28	Hutton Roof	1,669	10.2	13.3	0.18	0.007	57
29	St Bees (Rottington)	953	5.4	9.5	0.30	0.015	18
30	Harrington	923	8.7	11.4	0.22	0.011	40

The uncertainties in analysis are equivalent on average to coefficients of variation of for ^{137}Cs 8%, $^{239+240}$Pu 4% and ^{238}Pu 20%.
1 pCi = 10^{-12} Ci = 37 mBq.

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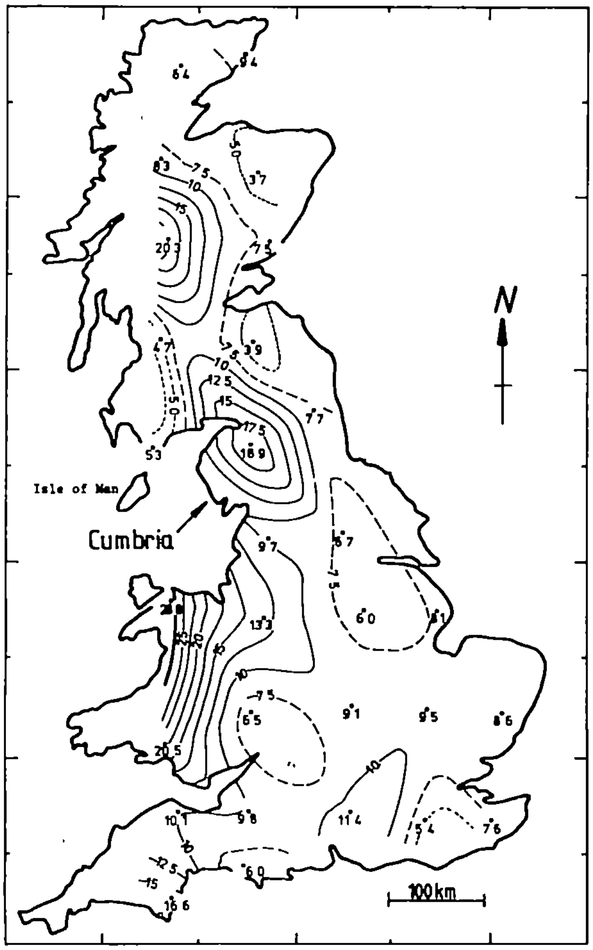

Fig. 1 Cumulative deposition of ^{137}Cs to grassland in Britain (1977). pCi cm^{-2} to 30 cm depth.

The radioactivity discharged by 1978 from Sellafield into the Irish Sea includes more than 12 kCi of Pu α activity and 600 kCi of ^{137}Cs (see ref. 4).

The investigation described here fell into three parts: (1) a survey of radioactive deposits accumulated in soil throughout Cumbria—an intensification of the larger scale survey of Britain; (2) a more detailed survey of soil along the coastal region, together with transects running inland from the coast; (3) measurement of current levels of radionuclides in air and rain.

Cumbria soil survey

The area of some 2,500 km^2 in Cumbria[5] (and the Isle of Man) was covered by a network of sampling sites based on a grid of 10 km side. Soils were sampled in 1978 to a depth of 30 cm from permanent grassland and woodland. Sites were selected on undisturbed land, that was likely to remain undisturbed to allow repetitive sampling in the future and with no history of flooding.

Samples of soil were taken with a corer of diameter 3.8 cm. At each site at least 10 cores were taken at random at both 0–15 cm and 15–30 cm depth and included the grass and root-mat layer. The cores were bulked to provide composite samples for each depth. The composite samples were air dried (at room temperature) and ground to $<150\ \mu$m, including stones and roots. About 100 g of soil were analysed for ^{137}Cs and Pu. The content of ^{137}Cs was determined by γ-ray spectrometry using a Ge–Li detector. Plutonium was leached from the soil by a 5:1 mixture of HNO_3/HF using ^{242}Pu as an internal tracer. $^{239+240}$Pu and ^{238}Pu were separated from the leachate and analysed by α spectrometry.

The accumulated depositions of ^{137}Cs and Pu are listed in Table 1 in terms of radioactivity per unit area of ground surface. As mentioned previously these deposits include a contribution from nuclear weapon fallout: the measured deposit relative to weapon fallout is shown in Fig. 2 for the two radionuclides, by converting the results of Table 1 as follows. Average deposition (0–15 cm) from weapon fallout in Britain as determined by the countrywide soil survey is 7.5 pCi cm^{-2} for ^{137}Cs and 0.14 pCi cm^{-2} for $^{239+240}$Pu (ref. 3), both normalized to 1,000 mm annual rainfall. (The coefficients of variation are respectively 50 and 25% for 58 sites.) The characteristic ratio in deposition of these radionuclides in 1977 is therefore about 54. Normalization to annual rainfall rests on the effective proportionality between weapon fallout and rain demonstrated in the countrywide survey[3], and on earlier work[6]. The average rainfall for the period 1941–70 was extracted from an index[7] of the rainfall stations nearest to the soil sampling sites. The relative deposition is then given by the ratio of the observed deposit to the nuclear weapon deposit scaled to the mean annual rainfall at the sampling site. Contours of relative rather than absolute deposition are mapped for ease of presentation. The finer structure exhibited by more intensive sampling is not shown. There are uncertainties in the derivation of the relative deposition values, associated with the interpolation between raingauge stations, retention of ^{137}Cs and Pu in the top 15 cm of soil and normal variability in sampling and analysis. Hence only values of relative deposition in excess of 1.5 are considered to be significantly greater than weapon fallout.

Figure 2*a* shows that the deposition of ^{137}Cs is concentrated near the coast and decreases fairly rapidly inland where the levels are indistinguishable from those due to weapon fallout. The major pattern located around Sellafield represents an accumulation of ^{137}Cs released presumably by aerial emission. To the south-east a smaller peak can be attributed to ^{137}Cs that escaped in the Windscale accident of 1957 (ref. 8). The results for each site (Table 1, Fig. 2) are compounded from the analysis of 10 cores. The relative deposition at site no. 6 of ^{137}Cs is consistent with the results of the Bootle transect[9].

Figure 2*b* shows that the contours of relative deposition of $^{239+240}$Pu are similarly concentrated around Sellafield, with the hinterland mainly free of excess radioactivity. However, there is no anomaly to the south-east, confirming the absence of measurable amounts of this radionuclide from the emission during the accident in 1957. Another difference from the ^{137}Cs pattern is indicated by the slight enhancement along the coast remote from Sellafield, suggesting that $^{239+240}$Pu has arrived from the seaward direction. This indication is supported by the values of the ratio ^{137}Cs/$^{239+240}$Pu (Table 1), measured at sampling sites near the coast, that are appreciably lower than the values for the hinterland (which are characteristic of weapon fallout). This implies that there is a relative excess of $^{239+240}$Pu along the coast not only in the vicinity of Sellafield but also in the more distant parts. In the Isle of Man no significant deposits in soil of ^{137}Cs and $^{239+240}$Pu were found in excess of weapon fallout[5], because presumably of the greater distance from the point of discharge to the sea.

It is possible to integrate the various depositions to grassland soils in Cumbria over the study area as defined by Fig. 2. Thus the excess of $^{239+240}$Pu (to 15 cm depth) due to the Sellafield operation is 2.4 Ci against a background due to weapon fallout of 5.9 Ci: the corresponding estimates for ^{137}Cs are 54 Ci against 315 Ci. The uncertainty of these integrals is probably less than a factor of two.

The radiological implications of these radionuclides in soil may be assessed by considering the potential for resuspension—by wind or other mechanical disturbance—and subsequent inhalation by man. The uppermost layers would be involved in resuspension, whereas most of the deposition over years will have penetrated more deeply into the soil. Calculations of this type have shown that the highest concentrations observed in soil in Cumbria are a very small fraction of the 'derived limit'[5] and hence constitute an insignificant hazard. This limit is derived from the limits set by the International Commission on Radiological Protection for the protection of the general public.

Table 2 Radionuclides in airborne and deposited material (November 1978–October 1979)

	Ratio of coastal station to weapon fallout			Ratio of coastal to inland station			Nuclear* weapon fallout
	St Bees	Eskmeals	Walney	St Bees	Eskmeals	Walney	
Air							(10^{-18}Ci kg^{-1})
^{137}Cs	9.2	4.9	3.3	0.9	0.8	1.2	240
$^{239+240}$Pu	12	19	4.0	1.8	2.7	1.9	6
^{241}Am	80	190	30	1.6	9	2.0	0.2
Na (stable)	—	—	—	2.5	1.9	1.3	—
Deposit							(10^{-15}Ci cm^{-2})
^{137}Cs	10.7	8.9	4.7	2.5	2.0	1.0	36
$^{239+240}$Pu	120	240	56	7.1	14	4.6	0.4
^{241}Am	1,000	1,600	600	10	16	6	0.019
Na (stable)	—	—	—	9.4	5.0	1.7	—

* As observed at Milford Haven[14].

Coastal survey and transects

As well as the comprehensive survey of Cumbrian soil, a more rapid and detailed study was made of the coastal region together with transects running inland from the coast. The coastal and transect surveys[9] were designed to give an early indication of any radioactive anomalies and also to discover any effect due to the radioactivity discharged from Sellafield into the Irish Sea.

The sampling method was similar to that described above except that the samples were restricted to the top 15 cm of soil. Samples were taken from grassy sites, generally ~2 km apart, from roadside verges, edges of fields and open moorland between December 1977 and March 1978. Six transects were selected, starting at points on the coast between 10 km north (St Bees) and 30 km south (Kirkby) of Sellafield. The transects extended inland to 20 km: samples were collected at various times between 1977 and 1979. The samples were analysed for ^{137}Cs and Pu isotopes by methods described above: a limited number were also analysed for ^{241}Am.

The results from the 45 central sites in the coastal survey are shown in Fig. 3, together with the ratios ^{137}Cs/$^{239+240}$Pu and ^{238}Pu/$^{239+240}$Pu. The weapon fallout baselines for each parameter are shown as straight lines, because the rainfall is effectively uniform over the coastal region.

Figure 3 shows that there are significant departures from the baseline for all parameters. All sites between 13 km north and 27 km south of Sellafield have excess $^{239+240}$Pu over the baseline value (that is >1.5 times). The highest value is 70 times and there are some anomalous values at greater distances. The value

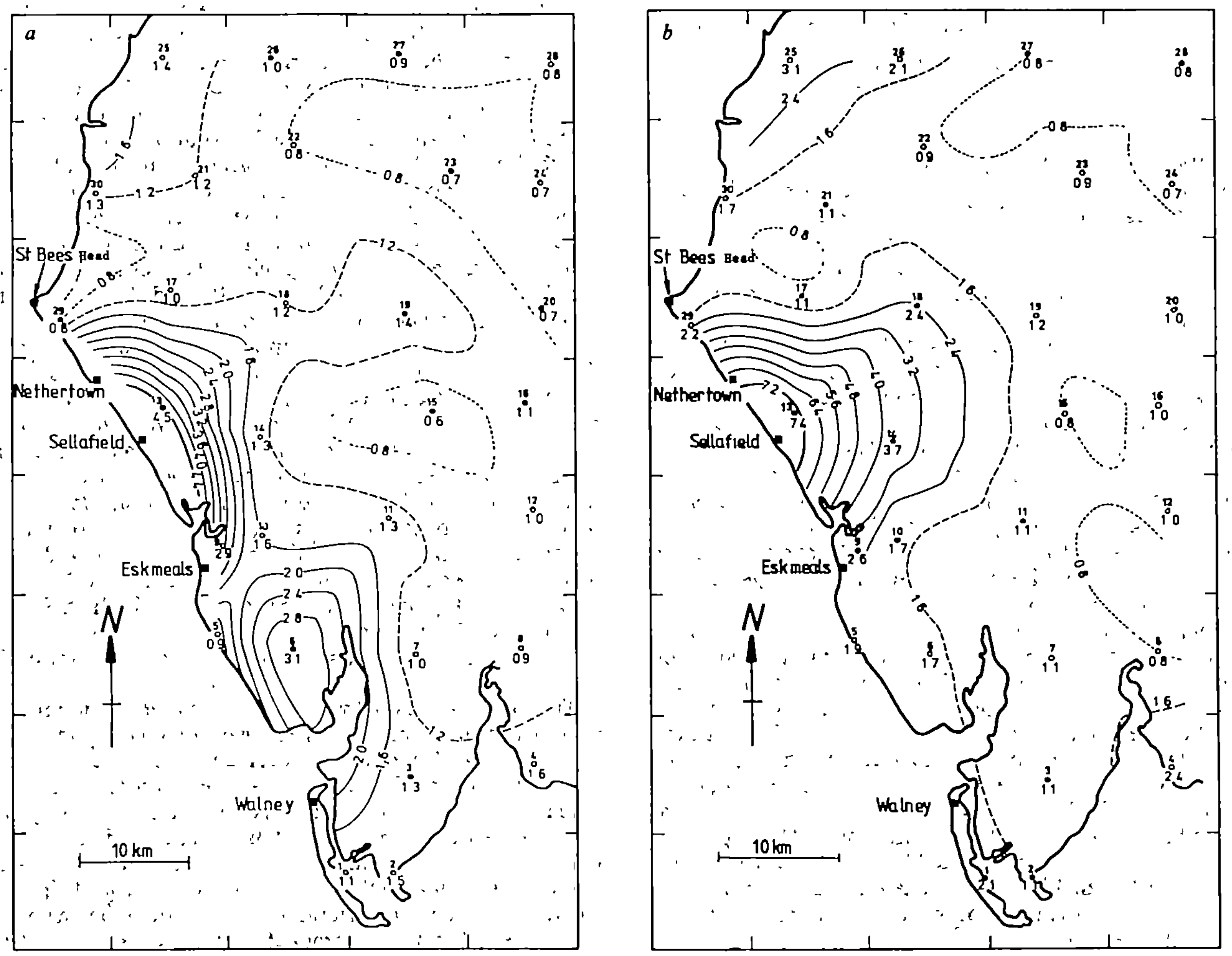

Fig. 2 Cumulative deposition of *a*, ^{137}Cs; *b*, $^{239+240}$Pu in Cumbria grassland (1978) relative to nuclear weapon fallout: 0–15 cm depth. Upper number is site identifier.

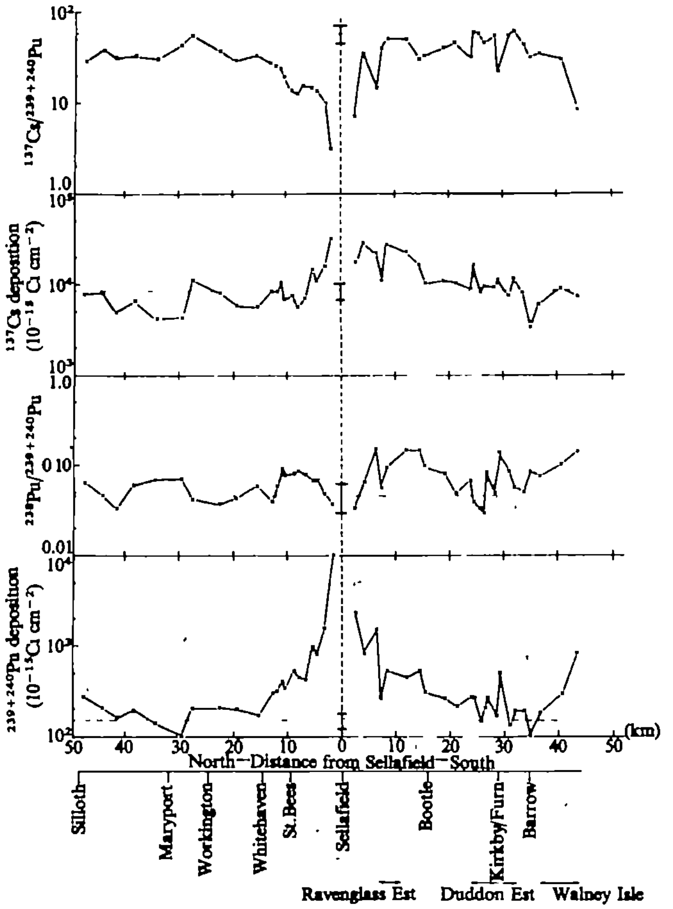

Fig. 3 Cumulative deposition of plutonium and ^{137}Cs in coastal soil of Cumbria (1977–78): 0–15 cm depth. – – –, Weapon fallout baseline.

of the ratio $^{238}Pu/^{239+240}Pu$ is elevated over the central region except close to Sellafield where it is depressed below the baseline. The deposition of ^{137}Cs does not exceed the baseline by as much as does Pu, in fact throughout the survey the ratio $^{137}Cs/^{239+240}Pu$ tends to lie below the baseline. However, the asymmetry of both radionuclides, with more to the south, is greater for ^{137}Cs.

The transect measurements show enhanced values of $^{239+240}Pu$ at the coast, between 3 and 11 times the weapon fallout baseline[9,10]. These fall to the baseline value at ~5 km and further inland, and the ratio $^{238}Pu/^{239+240}Pu$ is increased within 5 km of the sea above the value 0.05 which is characteristic of weapon fallout[11]. On the other hand there is no marked enhancement in the deposition of ^{137}Cs along the transects.

The patterns displayed by the coastal and transect surveys may be interpreted as follows. The excess ^{137}Cs can be explained by routine atmospheric discharges from Sellafield, together with the accident in 1957. Some of the excess local plutonium can be attributed to atmospheric discharges but it is difficult to use this source to account for the more remote coastal deposition (in excess of weapon fallout) and in particular for the regular decrease along the transects with increasing distance from the coast. These features suggest a contribution of Pu, but little ^{137}Cs, from the seaward direction. This contribution is marked by an enhanced value for the ratio $^{238}Pu/^{239+240}Pu$ approaching the value of ~0.3, characteristic of Sellafield discharges[12], at the coast but merging into the baseline value, of 0.05 due to weapon fallout[11], at ~5 km inland. The reduced values of this ratio close to Sellafield can be related to early atmospheric discharges[13] after short times of irradiation of the fuel, that is before substantial build-up of ^{238}Pu.

A two-dimensional summation of accumulated radionuclides may be based on the coastal and transect results, but neglecting the data from sites within 3 km of Sellafield: these were probably affected by direct atmospheric discharges. This rough estimate suggests that 1–2 Ci of excess Pu has been deposited in a coastal strip about 5 km wide and 40 km long. The deposit represents 10^{-4} of the quantity discharged to sea[4].

Airborne and deposited material

So far we have concentrated on an investigation of the cumulative deposition of radionuclides in soil. The coastal survey had shown anomalous accumulation at sites some distance from Sellafield: a complementary series of measurements was made of the current levels of radionuclides in air and rain. Six stations were established in the Cumbrian coastal region, deployed as three pairs, one of a pair on the coast and the other several kilometres inland. The coastal stations were sited at St Bees Head, Eskmeals and Walney (Fig. 2), where an excess of cumulative plutonium had been demonstrated (Fig. 3).

Airborne particulate was sampled continuously at a height of 1 m above ground by passing air through polystyrene filters at the rate of ~75 tonnes per month. Deposited material (wet or dry) was collected in a plastic bottle (25 l), containing tracer and carrier solution, through a funnel of 40 cm diameter. The monthly samples of airborne dust on filters and of rainwater (after evaporation and including the insoluble material) were analysed by γ-ray spectrometry followed by radiochemical methods, whereby Pu and Am were separated, using ^{242}Pu and ^{243}Am as internal tracers, and measured by α spectrometry. Stable trace elements were determined, as appropriate, usually by neutron activation analysis.

The results of these measurements in terms of the annual mean values and monthly ranges for the period November 1978 to October 1979 are reported in detail elsewhere[14] and are available for several radionuclides (actinides and fission products) and stable elements. For the present purpose the data are compressed into Table 2 which is restricted to the results for a few radionuclides and one stable element. These results of concentrations in airborne or deposited material are expressed as ratios to (1) contemporary values of weapon fallout observed at the long-established station at Milford Haven (distance 320 km) and (2) those from the corresponding inland stations. The information in Table 2 demonstrates: (1) an excess of radionuclides compared with nuclear weapon fallout; (2) an excess at the coast compared with inland (except for ^{137}Cs in air); (3) a greater excess for actinides than for fission products (particularly for deposited material); and (4) an apparent difference in the behaviour of airborne and deposited material.

The excess over nuclear weapon fallout is most marked for the actinides in deposited material (Table 2). Most of the additional ^{137}Cs can be explained by contributions from the discharges to air at Sellafield and from seawater that is suspended as spray[14]. In the case of actinides the same explanation cannot apply. Most of the actinides come from the seaward direction but if there is an association with suspended seaspray then a large enrichment over normal seawater is required[14], by a mechanism that is under investigation. This required enrichment is indicated by the ratios of the radionuclides to sodium compared with those in bulk seawater from the Irish Sea. The station at St Bees Head is 100 m above sea level and is likely to sample other forms of spray than the other coastal stations at sea level.

The discrepancies between airborne and deposited material in respect of the comparisons with weapon fallout and between coast and inland suggest an instrumental effect due to particle size, based on the postulate that a much larger fraction of the actinides than fission products is carried by relatively large airborne particles. This is qualitatively in accordance with the inference above that the main source of Pu and ^{241}Am is suspension of material coming from the seaward direction, whereas the major contribution to ^{137}Cs (together with ^{134}Cs and some other fission products) comes from the Sellafield discharges to air. In some investigations sea-spray and sand particles have been observed airborne with diameters of 100 μm or greater[15,16]. The sampler used for collecting airborne particulate, like all similar equipment, has a reduced efficiency in field conditions for collecting particles above ~20 μm diameter especially in lateral winds of high speed. The same limitation does not apply to the deposition sampler: this explains the discrepancy between the results obtained for airborne and deposited material exhibited in Table 2.

The potential radiological hazard from airborne radioactivity may be estimated by calculating the derived air concentrations for inhalation of $^{239+240}$Pu, which is the critical radionuclide. (The 'derived air concentration' may be defined by analogy with the definition of the derived limit.) The highest annual average concentration observed was 93×10^{-18}Ci kg^{-1} of air at Eskmeals, representing 0.2% of the derived air concentration[14], modified for the public.

Sea to land transfer

In the general soil survey of Cumbria it was shown (Fig. 2*b*) that there was a slight but significant enhancement of $^{239+240}$Pu in coastal regions remote from Sellafield. This indication is supported by the values (Table 1) of ^{137}Cs/$^{239+240}$Pu. The ratio is appreciably lower at the coast than that inland, which is characteristic of weapon fallout. This implies an excess of $^{239+240}$Pu at the coast and is consistent with a source of the excess lying in a seaward direction. Along the transects running inland the concentration of $^{239+240}$Pu was enhanced at the coast and decreased with increasing distance from the coast[9,10]. This feature suggests a significant contribution of $^{239+240}$Pu (and ^{241}Am) but not ^{137}Cs from the sea: the excess is characterized by values at the coast of the ratio ^{238}Pu/$^{239+240}$Pu that are higher than the baseline due to nuclear weapon fallout and nearer to that of the Sellafield discharges to sea[12]. For the airborne and deposited material the sampling and analysis of current levels at a time of very low values of contemporary weapon fallout will provide a more sensitive comparison with the baseline than does a comparison of the corresponding accumulations of the radionuclides in soil. In this case the comparison showed an excess of radionuclides at the coast compared with inland (associated with sodium from sea) as well as an excess over nuclear weapon fallout (Table 2). The extra ^{137}Cs could be attributed to sea-spray containing the concentration of seawater from the Irish Sea. For the actinides Pu and ^{241}Am, it is necessary to seek a mechanism of enrichment to explain the higher concentrations relative to sodium.

The radioactivity discharged by 1978 from Sellafield into the Irish Sea includes more than 12 kCi of Pu α activity and 600 kCi of ^{137}Cs (ref. 4). Most of the ^{137}Cs remains in solution whereas about 95% of the plutonium is removed to sediment[17]. The evidence summarized above points to a mechanism of physical transfer to land of a very small fraction of the radionuclides in the sea. Possible mechanisms are (1) suspension of the sea surface in the form of spray, either directly by wind or by bubble bursting, (2) injection of seawater and sediment into the air by waves breaking in the surf zone and (3) movement of sediment to intertidal regions and subsequent resuspension by wind. The second possibility, that involves the resuspension of sediment within the sea before release as enriched sea-spray, would satisfy the condition of enrichment that is required for the insoluble $^{239+240}$Pu (and perhaps ^{241}Am). In the case of the soluble ^{137}Cs there would be no enrichment. It has been shown that other means of enrichment are possible, for example, of the sea surface microlayer with stable trace elements[18,19]. More detailed work[20] has been started to elucidate the mechanism of sea-to-land transfer of these radionuclides in order to estimate the magnitude of the effect over the long term.

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Effects of a cloned cell line with NK activity on bone marrow transplants, tumour development and metastasis *in vivo*

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Natural killer (NK) cells cloned in vitro *have been transferred into NK-deficient hosts. These cells have been shown to have a role in the rejection of allogeneic bone marrow grafts, resistance to both radiation-induced thymic leukaemia and challenge with melanoma tumour cells. It appears that NK cells have an important role in immune surveillance.*

THE low incidence of spontaneous tumours in congentially immunodeficient athymic 'nude' mice[1], which lack functional T cells has raised questions about the role of conventionally induced cytotoxic T lymphocytes (CTL) in the immunosurveillance of tumour formation. Attention has therefore been focused on 'so-called' nonspecific cellular mechanisms such as natural killer (NK) cells[2]. NK cells, which are present in 'nude' as well as normal mice, can lyse certain tumour cells *in vitro* spontaneously, and therefore may be an important primary line of defence against neoplastic formation *in vivo*[3]. The low frequency of NK cells in lymphoid organs and the lack of well-defined and unique NK cell surface markers have made

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the isolation and study of NK cells rather difficult but recently, murine NK cells have been established and cloned as permanent cell lines *in vitro*[4]. Such NK clones provide a powerful tool with which to explore the *in vivo* functions of these important and interesting cells.

Here we report the *in vivo* effects of an NK cell clone in three experimental animal models. The first involves the rejection of allogeneic bone marrow transplants, the second the inhibition of tumour metastasis, and the third the suppression of radiation-induced thymic leukaemia. First, we show that NK cell-deficient C57BL/6–beige mice[5,6], injected with cloned histocompatible NK cells, develop the ability, comparable to that of normal C57BL/6 mice, to reject allogeneic bone marrow grafts. The bone marrow rejection is specific for target structures primarily encoded by genes in the H–2D region of the murine major histocompatibility complex (MHC). Second, using the lung colony assay, we show that lung implantation of intravenously injected B16 melanoma tumour cells in cyclophosphamide-treated (that is, NK-deficient) C57BL/6 mice is markedly inhibited in those mice previously injected with syngeneic NK cells. Third, we demonstrate that the incidence of radiation-induced thymic leukaemia in C57BL/6 mice is dramatically reduced by intravenous administration of syngeneic cloned NK cells 3 months before the normal onset of thymic leukaemia. Taken together, these results support a role for NK cells in bone marrow graft rejection and in the surveillance of neoplasia.

Cloning of NK cells

As C57BL/6 mice were used to study the potential *in vivo* function of NK cells we have established and cloned NK cell lines from C57BL/6 mice for these experiments as previously described for BALB/c and BALB/c *nu/nu* cell clones[4,7]. C57BL/6 splenocytes were cultured in complete RPMI 1640 medium supplemented with concanavalin A (Con A) conditioned medium[4]. Cell cultures exhibiting NK activity were cloned by limiting dilution and individual isolates assayed for NK activity on a panel of NK-sensitive and resistant tumour targets[7]. The results showed that the cytolytic pattern of these clones was characteristic for NK cells and identical to that previously described for BALB/c NK clones. The cell-surface marker phenotype of the C57BL/6 NK clones was similar to that previously associated with cloned BALB/c NK cell lines[7], namely Thy 1.2$^+$, T200$^+$, Lyt 1$^-$, Lyt 2$^-$. The presence of dense granules imaged by electron microscopy was also characteristic of these C57BL/6 clones (manuscript in preparation). In the experiments described below, the C57BL/6 cell clone NK B61A2 was used.

Rejection of bone marrow grafts

Previous studies had shown that several positive correlations exist between NK activity *in vitro* and natural resistance *in vivo* to bone marrow transplants[8]. As C57BL/6–beige mice are genetically NK-deficient, they were used to study the potential role of cloned NK cells in the rejection of allogeneic marrow grafts. In a similar model, C57BL/6 mice were made NK-deficient by exposure to four, weekly 175-rad doses of γ-irradiation[9]. Both groups of NK-deficient mice were injected intravenously with 2×10^6 cloned NK B61A2 cells and 7 days later exposed to a lethal dose of γ-irradiation (850–950 rad) followed by transplantation with 2×10^6 allogeneic BALB/c bone marrow cells. Proliferation of donor-derived cells in the spleen (that is, CFU–S formation), indicative of repopulation of the lymphomyeloid system, was assessed 5–12 days after marrow transplantation by incorporation into splenocyte DNA of injected ^{125}I-labelled 5-iodo-2′-deoxyuridine (^{125}IUdR)[10,11]. Splenic repopulation by transplanted marrow cells was expressed as a percentage of retained radioactivity in the spleen. Results (Table 1*a*) show that normal C57BL/6 (H–2^b) mice reject BALB/c (H–2^d) bone marrow (that is, <0.05% ^{125}IUdR uptake), whereas beige mice and split-dose irradiated C57BL/6 mice are unable to reject allogeneic marrow transplants (that is, ^{125}IUdR uptake >0.5% which is equivalent to the syngeneic growth control of BALB/c marrow transplanted into irradiated BALB/c recipients).

In contrast, beige mice and split-dose irradiated C57BL/6 mice, previously injected with H–2 compatible cloned NK B61A2 cells, are capable of rejecting BALB/c bone marrow. Similar results were obtained in experiments assayed 12 days after marrow transplantation. A dose of 2×10^6 NK B61A2 cells was sufficient to cause complete marrow graft rejection in all injected animals when NK cells were administered between 2 and at least 30 days before marrow transplantation. However, when NK cells were administered shortly before marrow transplantation, the percentage of NK-injected mice able to reject marrow grafts was dependent on the dose of NK cells (data not shown).

Table 1 Rejection of allogeneic bone marrow by NK-deficient C57BL/6 mice injected with NK B61A2 cells

a

Recipient strain	Bone marrow donor	% ^{125}IUdR splenic uptake
BALB/c	BALB/c	1.36±0.3
C57BL/6	BALB/c	0.01±0.03
C57BL/6 (4x-175r)	BALB/c	0.81±0.04
C57BL/6 (4x-175r) +NK B61A2	BALB/c	0.01±0.01
C57BL/6 bg/+	BALB/c	0.02±0.01
C57BL/bg/bg	BALB/c	1.43±0.1
C57BL/6 bg/bg +NK B61A2	BALB/c	0.05±0.01

b

Bone marrow donor	H–2 haplotype									% ^{125}IUdR splenic uptake in irradiated H-2^b recipients		
	K	IA	IB	IJ	IE	IC	S	G	D	*bg/bg*	*bg*	*bg/bg* + NK B61A2
BALB.B	b	b	b	b	b	b	b	b	b	1.33±0.10	1.13±0.30	1.17±0.05
BALB/c	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	1.73±0.30	0.02±0.01	0.05±0.01
B10	b	b	b	b	b	b	b	b	b	0.76±0.20	1.57±0.10	1.42±0.30
B10.D2	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	0.49±0.04	0.01±0.001	0.01±0.001
B10.A(18R)	b	b	b	b	b	b	b	b	*d*	0.51±0.10	0.01±0.01	0.03±0.01
B10.HTG	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	b	0.62±0.10	0.21±0.01	0.19±0.02
D2.GD	*d*	*d*	*d*	b	b	b	b	b	b	0.81±0.10	0.11±0.06	0.19±0.03

a, Normal C57BL/6, C57BL/6–beige (*bg/bg*), or C57BL/6 mice treated with four weekly doses of 175 rad γ-irradiation were used as bone marrow graft recipients. Shortly before bone marrow cell transfer, recipient mice were exposed to a lethal dose (850 rad) of ^{60}Co γ-irradiation. Donor marrow cells (2×10^6 cells per mouse) were injected intravenously into sex- and age-matched recipients. Proliferation of transplanted cells was assessed 5 days later by measuring the incorporation of ^{125}IUdR into splenocyte DNA[10,11]. Uptake of radioactivity was expressed as a percentage of retained radioactivity in the spleen. The geometric mean and standard error of the arithmetic mean for each group of mice (3–6 animals) are given. Irradiated recipients, syngeneic to the donors, were used as positive controls for uninhibited bone marrow growth (consistently $\geqslant$0.50% splenic ^{125}IUdR uptake). Irradiated mice not injected with marrow cells were used as negative controls for ^{125}IUdR incorporation (<0.05% splenic uptake). Groups of mice injected with NK cells received 2×10^6 cloned C57BL/6 NK B61A2 cells intravenously 7 days before bone marrow transplantation. The data presented are from experiments assayed on day 5; however, 12-day assays showed similar results. *b*, Marrow graft transplantation into C57BL/6–beige and C57BL/6-*bg*/+ recipients was done as in *a*, using bone marrow cells obtained from various H–2 recombinant mouse strains possessing *d* alleles at different regions within the H–2 gene complex. Some *bg/bg* mice were injected intravenously with 2×10^6 cloned NK B61A2 cells 7 days before lethal body irradiation and marrow transplantation (*bg/bg*+NK B61A2). Marrow growth control values, obtained by injecting donor cells into histocompatible recipients, were comparable to those values obtained by injecting cells from the same preparation into *bg/bg* recipients. C57BL/6 and C57BL/6 *bg*/+ recipients showed identical rejection patterns of bone marrow transplants.

Table 2 Effect on allogeneic marrow grafts of NK B61A2 cells and cell lines without NK activity

Recipient	Cell lines used for injection	NK activity of cell lines	Bone marrow donor	% Splenic ^{125}IUdR uptake
a (C57BL/6×C3H)F_1	—		C57BL/6	0.01±0.001
(C57BL/6×C3H)F_1-*bg/bg*	—		C57BL/6	0.52±0.1
(C57BL/6×C3H)F_1-*bg/bg*	NK B61A2	+	C57BL/6	0.72±0.1
(C57BL/6×C3H)F_1-*bg/bg*	BC1	–	C57BL/6	0.95±0.02
(C57BL/6×C3H)F_1	–		C3H	2.0±0.4
(C57BL/6×C3H)F_1-*bg/bg*	–		C3H	2.7±0.02
(C57BL/6×C3H)F_1-*bg/bg*	NK B61A2	+	C3H	2.3±0.3
(C57BL/6×C3H)F_1-*bg/bg*	BC1	–	C3H	2.9±0.6
(C57BL/6×C3H)F_1	—		BALB/c	0.02±0.002
(C57BL/6×C3H)F_1-*bg/bg*	–		BALB/c	1.69±0.31
(C57BL/6×C3H)F_1-*bg/bg*	NK B61A2	+	BALB/c	0.21±0.06
(C57BL/6×C3H)F_1-*bg/bg*	BC1	–	BALB/c	1.93±0.09
b C57BL/6	–		BALB/c	0.03±0.01
C57BL/6-*bg/bg*	–		BALB/c	1.42±0.15
C57BL/6-*bg/bg*	NK B61A2	+	BALB/c	0.02±0.01
C57BL/6-*bg/bg*	BC1	–	BALB/c	1.05±0.06
C57BL/6-*bg/bg*	BB1	–	BALB/c	1.46±0.05
C57BL/6-*bg/bg*	CTLL-2	–	BALB/c	1.33±0.20

Bone marrow transplantation into irradiated normal and beige (C57BL/6×C3H)F_1 hybrid (*a*) or C57BL/6 (*b*) recipients was done as described in Table 1 legend using a 7-day assay. Some groups of mice were injected intravenously 7 days before marrow transplantation with 2×10^6 cells from various cell lines maintained in Con A conditioned medium which either express (+) or do not express (–) NK activity *in vitro* on YAC-1 target cells. Injected cell lines are the following: NK B61A2, a cloned cell line derived from C57BL/6; CTLL-2, a cell line derived from a C57BL/6 anti-BALB/c (H-2^b anti-H-2^d) mixed lymphocyte reaction[20]; BB1 and BC1, cell lines derived from C57BL/6–beige and (C57BL/6×C3H)F_1 mice, respectively, by culturing lymphoid cells in Con A conditioned medium. None of the cell lines, except NK B61A2, expresses NK activity. Growth control values for donor bone marrow were equivalent to those obtained in beige recipients and are not included.

As the phenomenon of marrow allograft rejection exhibits a unique specificity for antigens primarily encoded in the H-2D region[10–13] and to a lesser extent the H-2K region[14] of the MHC, it was important to determine the specificity of marrow graft rejection caused by cloned NK cells. NK cell-injected C57BL/6–beige mice were transplanted with bone marrow obtained from B10 and other congeneic mouse strains in order to map the genetic specificity of graft rejection. Results (Table 1*b*) show that NK-injected beige mice reject BALB/c (H-2^d) but not BALB.B (H-2^b) bone marrow which shows that H-2 encoded target determinants are responsible for rejection. Furthermore, when H-2 recombinant mouse strains are used as bone marrow donors, NK-injected beige mice reject transplanted marrow from donors possessing the H-2D^d allele [B10.A(18R)]. In contrast, relatively weak rejection of marrow cells expressing H-2K end disparity (B10.HTG and D2.GD) is observed[14], whereas H-2 identical bone marrow (B10) is not rejected. Several important conclusions can be drawn from these results: (1) a cloned NK cell line injected into syngeneic NK-deficient mice has an effect *in vivo*; (2) this function is specific in that H-2 disparate, but not H-2 identical bone marrow transplants are rejected; and (3) rejection is primarily, but not exclusively, directed towards antigenic determinants encoded by the H-2D region.

These findings raise the question of whether the effects observed are specifically due to the action of cells expressing NK activity. To demonstrate this, several kinds of controls were done. First, NK B61A2 cells were tested in NK-deficient semi-allogeneic (C57BL/6×C3H)F_1 beige hosts. Interestingly, in this model, NK B61A2 cells did not cause rejection of either C57BL/6 or C3H marrow transplants, although they do cause a significant but weak rejection of BALB/c allografts (Table 2*a*). Second, cell lines which do not express NK activity, but require Con A conditioned medium for their growth, do not cause bone marrow rejection. This is shown for several different cell lines. Cell line BC1, obtained from (C57BL/6×C3H)F_1 mice, grown in Con A conditioned medium and lacking NK activity, when injected into NK-deficient (C37BL/6×C3H)F_1 beige mice, does not cause rejection of C57BL/6, C3H or allogeneic BALB/c bone marrow (Table 2*a*). Similarly, cell line BB1, derived from C57BL/6-beige mice, which lacks NK activity, also fails to cause rejection of BALB/c bone marrow grafts when injected into C57BL/6–beige mice (Table 2*b*). Interestingly, the cell line CTLL-2, derived from a C57BL/6 anti-BALB/c (H-2^b anti H-2^d) mixed lymphocyte reaction[20], which also requires Con A conditioned medium for proliferation and expresses no NK activity, fails to cause bone marrow rejection (Table 2*b*). These results suggest that bone marrow rejection *in vivo* is caused by cell lines which express NK cytolytic activity *in vitro* and that cell lines merely grown in Con A conditioned medium are not sufficient to cause marrow allograft rejection.

An as yet unexplained observation is that the injection of NK B61A2 cells into (C57BL/6×C3H)F_1 mice leads to rejection of allogeneic BALB/c but not parental marrow grafts. Perhaps NK cells express exquisite *in vivo* H-2 specificity not obviously demonstrable *in vitro*, as NK B61A2 lyses tumour target cells and peritoneal exudate cells independent of the H-2 haplotype *in vitro* (unpublished results). Accessory cells and/or humoral factors may participate in the rejection of marrow grafts *in vivo*, as one or both components would be absent from NK cytotoxic assays *in vitro*. It is important in this respect that NK B61A2 exhibits antibody-dependent-cell-mediated lysis *in vitro* (unpublished results). Therefore, the

Table 3 Suppression of lung tumour colony formation in NK-deficient mice injected with NK B61A2

Recipient mice	Cell-lines used for injection	NK activity of cell lines	B16/F10 tumour dose	Lung tumour colonies
a C57BL/6	–		1×10^5	54±14
C57BL/6-CY	–		4×10^5	485±72*
	NK B61A2	+	4×10^5	225±32
C57BL/6-CY	–		1×10^5	330±27
	NK B61A2	+	1×10^5	59±18
C57BL/6-CY	–		5×10^4	60±11
	NK B61A2	+	5×10^4	12±2
b C57BL/6	–		1×10^5	37±16
C57BL/6-CY	–		1×10^5	267±12
C57BL/6-CY	NK B61A2	+	1×10^5	43±18
C57BL/6-CY	CTLL-2	–	1×10^5	260±25
C57BL/6-CY	BC1	–	1×10^5	203±2

C57BL/6 mice injected intraperitoneally with cyclophosphamide (CY) (200 mg per kg) 4 days previously, were injected intravenously with various doses of B16/F10 melanoma cells[15]. Some mice were also given 2×10^6 cloned NK B61A2 cells, BC1 cells or CTLL-2 cells intravenously 3 h before tumour cell challenge. Pulmonary colonies were counted 3 weeks later after fixation in Bouin's solution and examination under a dissecting microscope. Mice were age- and sex-matched for each experiment. The mean and standard deviation of the number of tumour colonies per lung is presented.

* Mice given 4×10^5 tumour cells alone (*a*) died before the termination of the experiment so the number of tumour colonies in this group does not show a linear dose relationship. Experiments using C57BL/6–beige mice showed similar results

Table 4 Decreased incidence of radiation-induced thymic leukaemia in C57BL/6 mice injected with cloned C57BL/6 NK cells

Treatment		Cumulative thymoma incidence (mice with tumour/total no. of mice) Months post-irridadiation							
Irradiation	NK	3	4	5	6	7	8	9	% Incidence
–	–	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0
+	–	0/12	3/12	5/12	8/12	10/12	10/12	10/12	83
+	+	0/12	0/12	0/12	1/12	1/12	1/12	1/12	8

Groups of 4-week old C57BL/6 mice were exposed to four weekly doses (175 rad) of ^{60}Co γ-irradiation[19]. One week after the final irradiation, mice were injected intravenously with $2–3\times10^6$ cloned NK B61A2 cells on a weekly basis for 4 weeks. Mice were observed up to 9 months later, and at death, necropsy was performed to determine the incidence of thymic leukaemia. Presented data are from a representative experiment and show the cumulative number of mice with thymic leukaemia relative to the total number of animals.

failure of NK B61A2 to reject parental marrow grafts as opposed to allogeneic grafts could be a reflection of naturally occurring antibody (that is, anti-allo antibody) in the (C57BL/6 × C3H)F_1 mice.

An important and related question is whether injection of NK B61A2 cells into NK-deficient mice directly reconstitutes the animal's NK activity or whether other cell types involved in the mechanism of transplant rejection are recruited. Migration studies have shown that only about 1% of the injected NK cells eventually end up in the spleen, a number which is probably too small to be detectable by conventional cytotoxicity assays but may be sufficient to cause bone marrow rejection. Even after 9 days, only low NK activity is detectable in splenocytes of NK-injected mice. This indicates that NK reconstitution is incomplete and would argue against rapid NK cell proliferation or massive recruitment of host NK cells as a result of NK B61A2 injection.

Effect of NK cells on melanoma

Further experiments explored the role of NK cells in the surveillance of neoplasia. Recent studies have implicated NK cells in the control of experimental metastasis of the C57BL/6 melanoma, B16/F10 (refs 15–17). In this model, either C57BL/6–beige or C57BL/6 mice made NK-deficient by prior injection of cyclophosphamide (day 4, 200 mg per kg)[18] are injected with melanoma cells. Tumour-challenged NK-deficient mice develop pulmonary tumour colonies 2–3 weeks later. Using this system, we examined the effect of adoptively transferred cloned NK cells on the development of B16 tumour colonies in the lungs of C57BL/6 mice. Cyclophosphamide-treated mice were injected with 2×10^6 NKB61A2 cells 3 h before intravenous tumour cell injection and the number of melanoma tumour colonies growing in the lungs was assayed 3 weeks later. Results show that mice injected with NK cells exhibit a 50–85% reduction in lung tumour colonies compared with controls (Table 3*a*). Moreover, tumour colonies were not detected in other organs of NK B61A2 injected mice, whereas control mice frequently showed additional tumours in the liver. In contrast, injection of either CTLL-2 or BC1 cells, which lack NK activity, into NK-deficient mice did not result in a decrease in lung tumour colonies (Table 3*b*). Experiments using C57BL/6–beige mice showed similar results (data not shown). Hence, injection of cells with NK activity into NK-deficient mice appears to cause inhibition of pulmonary tumour colonies. This demonstrates that cloned NK cells are able to perform a function *in vivo* already suggested by previous studies[15–17] in which spleen cell preparations high in NK activity were used.

Radiation-induced thymic leukaemia

The *in vivo* suppressive effects caused by this clone of cells with NK activity on B16 melanoma growth led us to investigate the potential antitumour activity of cloned NK cells on the development of radiation-induced thymic leukaemia. In this model, 70–90% of C57BL/6 mice, which are subjected to four weekly doses of 175–200 rad of γ-irradiation beginning at 4 weeks of age, develop lethal thymic leukaemia 3–5 months after the last irradiation[19]. Recent studies have shown that this leukaemogenic split-dose irradiation also results in severe depression of NK activity[9]. Therefore, C57BL/6 mice were exposed to leukaemogenic split-dose irradiation and divided into two groups, one of which was given four weekly injections of cloned NK B61A2 cells ($2–3\times10^6$ per mouse) after the last irradiation and the other left as control for leukaemia development. Results collected over 9 months show that ~80% of the irradiated control mice developed thymic leukaemia, whereas less than 10% of the irradiated mice injected with syngeneic NK B61A2 cells developed thymoma (Table 4).

We have noted the following observations in experiments currently under way. The lowest incidence of leukaemia occurs in mice injected with NK cells immediately after the last irradiation. Later injection of NK cells (that is 2–3 months after irradiation), at a time when NK activity may slowly reappear, does not seem to prevent development of leukaemia, as is also the case with early injection of cell lines not expressing NK activity. These findings suggest that split-dose irradiated NK-deficient mice, transfused at the proper time with cloned histocompatible NK cells, may become resistant to the development of radiation-induced thymic leukaemia.

Conclusions

Taken together, our observations suggest that NK cells have a crucial role in the mechanism of marrow graft rejection. This in turn may reflect their potential regulatory role in stem cell development and differentiation. Furthermore, NK cells may play an important role in the control of neoplasia. Our experiments, however, do not resolve the question of whether the effects exerted by these cloned NK cells are mediated directly or indirectly. They show, however, the significance of lymphocyte cloning techniques for examining the functional activity of lymphocytes and may point toward experimental approaches in the control of neoplasia.

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A molecular map of the immune response region from the major histocompatibility complex of the mouse

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A stretch of 200 kilobases (kb) of DNA from the I *region of the mouse major histocompatibility complex has been cloned and characterized. It contains the genes for the biochemically defined class II proteins* E_α, E_β *and* A_β. *DNA blot analyses suggest that the* I *region may contain only 6–8 class II genes. Correlation of our molecular map with the genetic map of the* I *region confines two of the five* I *subregions,* I–J *and* I–B, *to less than 3.4 kb of DNA at the 3′ end of the* E_β *gene where a hotspot for recombination has been observed. Indeed, the* I–A *and* I–E *subregions may be contiguous. If so, the* I–B *and* I–J *subregions are not encoded in the* I *region between the* I–A *and* I–E *subregions.*

THE major histocompatibility complex (MHC) in mice encompasses about 2 centimorgans of DNA on chromosome 17 and encodes three different classes of proteins involved in immune responses[1,2]. Class I and class II molecules are both integral cell-surface glycoproteins, whereas class III molecules are serum components of the complement system. Class I molecules, typified by the transplantation antigens, serve as restricting elements for the response of cytotoxic T cells to virally infected cells. Class II molecules, also called *I*-region-associated or Ia antigens, are encoded in the *I* region of the MHC. They regulate the recognition of foreign antigens on the surfaces of macrophages and B cells by T cells[3]. These interactions lead to high or low immune responses to antigenic determinants controlled by the so-called *Ir* (immune response) genes. The Ia antigens appear to be equivalent to *Ir* gene products[4]. Initially it was believed that the *Ir* genes might encode the presumably vast array of T-cell receptor molecules[5]. However, recent studies using serological and protein analyses have suggested a much more limited number of *Ir* or *Ia* genes. A genetic map of the MHC is shown in Fig. 1.

The *I* region has been divided by recombinational analysis into five subregions: *I-A*, *I-B*, *I-J*, *I-E* and *I-C*[6]. The *I-A* and *I-E* subregions contain at least four genes encoding the Ia antigens. The two biochemically well characterized Ia antigens, I-A and I-E, are heterodimers composed of a 33,000 to 35,000-molecular weight α chain and a 28,000 to 31,000-molecular weight β chain and are expressed primarily on B cells and macrophages. The A_α, A_β and E_β chains are encoded in the *I-A* subregion whereas the E_α peptide is encoded in the *I-E* subregion. The *I-J* subregion appears to encode one or more soluble T-cell factors and cell-surface antigens on T cells and macrophages which suppress immune responses. Although these I-J suppressor factors have been serologically defined, their biochemical characterization is only beginning[7–9]. The *I-B* and *I-C* subregions are more controversial[4,6]. They were originally defined based on their apparent control of certain types of immune responses.

An important characteristic of the MHC is the extreme polymorphism of class I and class II molecules[10]. For example, there appear to be over 100 alleles each for the K and D genes of the class I category. Whereas the A_β and E_β loci also are very polymorphic, the A_α and E_α loci exhibit a limited degree of polymorphism. The constellation of alleles present in a particular MHC is called a haplotype. For example, the inbred mouse strain that we have studied, BALB/c, has a *d* haplotype MHC and expresses class I and class II chains denoted K^d, D^d, A^d_α, A^d_β, E^d_α, E^d_β, and so on.

Our initial studies on the genes of the major histocompatibility complex focused on the class I genes[11–13]. From a cosmid genomic library constructed from BALB/c sperm DNA, we isolated 54 class I clones which could be ordered into 13 clusters spanning 837 kb of DNA and contained 36 distinct class I genes[14]. This study demonstrated the effectiveness of cosmid vectors which incorporate very large eukaryotic DNA inserts (~40 kb) in the definition and linkage of MHC genes. Subsequently, we used the techniques of gene transfer to correlate many of these genes with their serologically defined gene products and have initiated studies into the analysis of their functions in cell–cell communication[15–17].

To extend this analysis to class II genes in the *I* region, we have used cDNA clones coding for human class II α and β chains[18,19] and using chromosomal walking procedures, have isolated about 200 kb of contiguous DNA from the *I* region of the inbred BALB/c mouse. Genes for three of the four biochemically defined class II chains, E_α, E_β and A_β, have been identified as has a fourth class II gene, denoted $E_{\beta 2}$, that has not been described previously. Correlation of our molecular map with the genetic map of the *I* region places these genes in the *I-A* and *I-E* subregions and confines the *I-B* and *I-J* subregions to less than 3.4 kb of DNA at the 3′ end of the E_β gene.

cDNA clones for human class II α and β chains

In man, the best known class II antigen is HLA-DR, which exhibits homology for *I-E* gene products of the mouse[20,21]. Other loci exist in man for different class II antigens, such as DC[22] (probably identical with DS[23]) and SB[24]. It appears that the human HLA-DC antigens are the equivalent of the mouse I-A antigens[23,25]. Several laboratories have reported the cloning and sequence analysis of cDNA complementary to human messenger RNAs encoding class II α and β chains[18,19,26–30]. Full-length cDNA clones for the α chain of HLA-DR were isolated from a cDNA library made from the human Raji cell line[18]. One of these clones, referred to as DR_α, is used here as a probe for the identification of the corresponding mouse genes.

Full-length cDNA clones for the polymorphic β chain of HLA-DR, as well as clones for another 'Ia-like' β chain, clearly distinct from HLA-DR, have been isolated from heterozygous and homozygous human cell lines[19]. The genes for these two different class II β chains show a high degree of polymorphism when analysed directly by Southern blot hybridization (C.W. *et al.*, in preparation). Our cDNA clones for this 'Ia-like' β chain are very similar to the β-chain clones reported by

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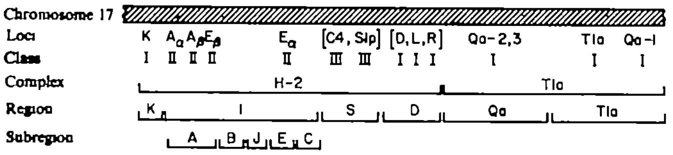

Fig. 1 Genetic map of the major histocompatibility complex of the mouse. The *K*, *D*, *L* and *R* genes encode the transplantation antigens which are found on all somatic cells, whereas the *Qa-2,3*, *Tla* and *Qa-1* genes encode class I molecules which are restricted in their expression to haematopoietic cells. The class III genes *C4* and *Slp* encode C4 components in the *S* region. Genes in brackets have not been mapped with respect to each other. Recombination frequencies are in centimorgans and taken from ref. 2. The centromere is located to the left.

others[27,29], and they are all clearly different from HLA-DR_β-chain genes[19,31]. As will be shown here, hybridization of this human 'Ia-like' β-chain gene to mouse DNA shows strongest homology with the A_β gene. Because the mouse A_α and human HLA-DC_α chains appear homologous[23,25], we feel that the 'Ia-like,' non-DR β chain is probably a component of the HLA-DC molecule. In this study, we therefore refer to this β-chain cDNA clone as DC_β.

Cloning of 200 kb of DNA from the *I* region

We isolated the mouse E_α gene by screening a cosmid genomic library previously constructed from BALB/c sperm DNA with the human DR_α-chain cDNA clone. This screen yielded the four overlapping cosmid clones 39.1, 17.2, 32.1 and 17.3 which defined a region of about 60 kb of DNA with the E_α gene in the middle (Fig. 2). We then isolated single-copy probes 4 and 8 from the 5′ and 3′ ends, respectively, of this 60-kb region and re-screened our cosmid library. Two overlapping clones (24.2 and 8.4) were obtained with probe 4 and eight overlapping clones (57.2, 52.4, 60.3, 21.1, 54.2, 16.2, 47.3 and 55.2) with probe 8. This chromosomal walking procedure was repeated once more with probes 2 and 9 which yielded the cosmid clones 41.1, 29.1 and 7.1 (Fig. 2). Finally, cosmid clone 34.2 was isolated by screening the library with the human DC_β-chain cDNA clone.

These 18 overlapping cosmid clones represent about 200 kb of DNA from the *I* region (Fig. 2). Although cosmid clones may delete certain DNA segments[14], there is no evidence for observable deletions since most of the DNA in this 200-kb region is represented by two or more independently generated cosmid clones. Independent evidence for the integrity of the chromosomal map was obtained by Southern blot analyses of BALB/c liver DNA cleaved with four different restriction

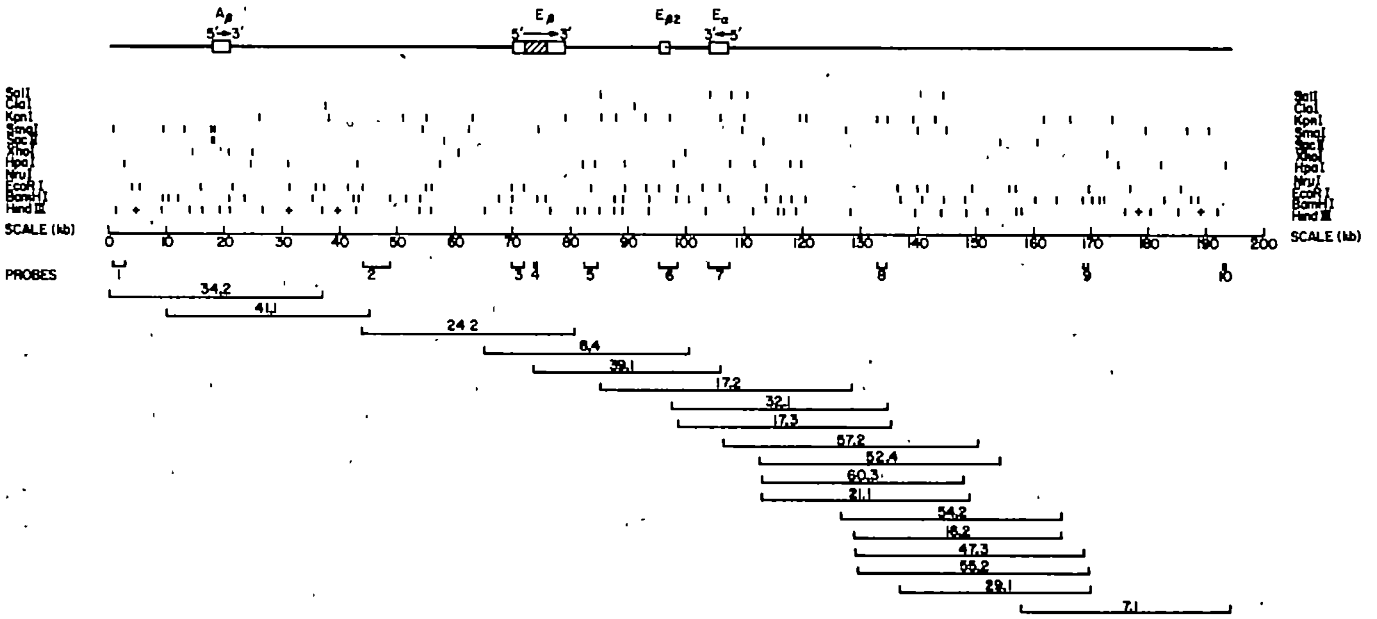

Fig. 2 Molecular map of 200 kb of the *I* region from the BALB/c mouse defined by 18 overlapping cosmid clones and containing four class II genes. Our cosmid BALB/c sperm DNA library, distributed on 66 nitrocellulose filters, contains about 250,000 colonies (representing three genome equivalents)[14]. It was hybridized sequentially with the DR_α-chain cDNA clone 39-1 prepared from human Raji cell line mRNA[18], probes 4 and 8 from cosmids 39.1 and 32.1, probes 2 and 9 from cosmids 24.2 and 55.2, and the DC_β-chain cDNA clone[19] isolated from Raji cell mRNA. For hybridization the *Pst*I fragments comprising the inserts of DR_α and DC_β (DR_α 39-1: 450 and 650 bp; DC_β: 350 and 800 bp in length; corresponding to the 5′ and 3′ part of the cDNAs, respectively) and the appropriate fragments from the cosmid clones (see below) were isolated by agarose gel electrophoresis and electroelution onto DE81 paper[44] and labelled by nick-translation to a specific activity of about $1-3\times10^8$ c.p.m. μg^{-1}. Hybridization was carried out for 16–18 h at 65 °C with a total of 10^5-10^6 c.p.m. ml^{-1} in 5×SSC, 10% dextran sulphate, 5× Denhardt's solution, 5 mM EDTA, 0.1% SDS, 150 $\mu g\ ml^{-1}$ denatured salmon sperm DNA and 1–2 $\mu g\ ml^{-1}$ *Hae*III-digested, denatured pBR322 DNA and 50 $\mu g\ ml^{-1}$ denatured BALB/c DNA to compete for contaminating restriction fragments containing pBR322 or repetitive mouse DNA sequences in the nick-translated probes. The filters were then washed at 65 °C twice for 30–60 min each in 3× SSC, 0.1% SDS and subsequently treated the same way in 2× SSC, 0.1% SDS when the screen was with the human cDNA clones, or in 1× SSC, 0.1% SDS when probes from the cosmid clones were used. Filters were exposed for 1–2 days with intensifying screens, positive colonies were picked from a replica set of the library kept frozen at −80 °C and purified by a second round of plating and hybridization as described previously[14] and above. For the isolation of the 18 overlapping cosmid clones, the same set of filters was used without any treatment between the individual hybridizations. To avoid picking of previously isolated cosmid clones, autoradiograms were compared with those from the last round of hybridization to identify only new cosmid clones. Plasmid DNA was then isolated from the cosmid clones as previously described and mapped with the restriction enzymes indicated by single and double digestions. A + between two restriction sites indicates the presence of more sites in this region for the same enzyme. The location and orientation of the class II genes shown as open boxes was determined by Southern blot analyses of restriction fragments from the cosmid clones with the separated 5′ and 3′ *Pst*I fragments from the human cDNA clones as hybridization probes. The sizes of the open boxes, therefore, give maximal lengths for the coding regions cross-hybridizing to our human probes. The hatched area in the E_β gene indicates the location of an intervening sequence presumably between the exons for the $\beta1$ and $\beta2$ domains[29,37] since the 5′ and 3′ probes from DC_β hybridize exclusively to the left and to the right of the intervening sequence, respectively (the internal *Pst*I site used to generate these probes is located around amino acid residue 90 at the border between the $\beta1$ and $\beta2$ domains). The 10 probes isolated from the 200-kb region were identified as being free of repetitive DNA sequences by their lack of hybridization to total mouse DNA as a labelled probe[45]. They were then shown to be composed of single-copy sequences by Southern blot hybridization against mouse genomic DNA. The 10 probes were isolated by agarose gel electrophoresis[44]. They represent the following restriction fragments: (1) 2-kb *Sma*I–*Hpa*I fragment from clone 34.2; (2) 4.7-kb *Eco*RI–*Bam*HI fragment from clone 24.2; (3) 2-kb *Eco*RI fragment from clone 24.2, subcloned into M13mp8; (4) 500-bp *Sau*3A fragment from clone 39.1 isolated from a 2.7-kb *Bam*HI fragment which extended into vector sequence; (5) 2.3-kb *Hin*dIII fragment from clone 39.1; (6) 3.2-kb *Eco*RI fragment from clone 8.4; (7) 3.4-kb *Sal*I fragment from clone 32.1, subcloned into M13mp8; (8) 1.6-kb *Kpn*I fragment from clone 32.1; (9) 800-bp *Sau*3A fragment from clone 55.2 isolated from a 2.9-kb *Bam*HI–*Nru*I fragment which extended into vector sequence; (10) 500-bp *Sau*3A fragment from clone 7.1 isolated from a 2-kb *Hpa*I–*Nru*I fragment which extended into vector sequence.

enzymes (*Eco*RI, *Bam*HI, *Kpn*I and *Hin*dIII) and hybridized with 10 single-copy probes scattered throughout the 200-kb region (Fig. 2 and below). In all cases the sizes of the genomic fragments agreed with those predicted by the map in Fig. 2. For example, Fig. 3 shows that probe 2 identified unique *Eco*RI, *Bam*HI, *Kpn*I and *Hin*dIII fragments in BALB/c DNA of 11, 6, 13 and 11.5 kb, respectively, which are in agreement with the restriction map established by the overlapping cosmid clones 41.1 and 24.2 (Fig. 2). These results confirm the small overlap between the two cosmid clones based on hybridization data and argues against major deletions or rearrangements even in this region, mainly defined by single cosmid clones.

The cloned *I* region contains four class II genes

It was expected that the human DR$_\alpha$-chain cDNA clone would hybridize to the mouse E$_\alpha$ gene because of N-terminal amino acid sequence homologies (refs 20, 21 and E. Sung *et al.*, in preparation) and known serological cross-reactions[32]. The gene identified in the middle of the 200-kb region with the human DR$_\alpha$ cDNA clone was subcloned and sequenced. The N-terminal amino acid sequence it encodes is 98% homologous to the E$_\alpha$ chain of the *k* haplotype (E. Sung *et al.*, in preparation) and the entire coding region is ~80% homologous to the human DR$_\alpha$ sequence at the DNA level[33]. We therefore believe that this class II gene is the mouse E_α^d gene and demonstrate below that the E$_\alpha$ gene maps into the *I-E* subregion.

As shown in Fig. 2, the E$_\beta$ gene was identified about 30 kb proximal to the E$_\alpha$ gene (the order of class II genes appears to be: centromere-A$_\alpha$-A$_\beta$-E$_\beta$-E$_\alpha$[34,35]). This class II gene was detected by hybridization to a chemically synthesized, 32-fold degenerate oligonucleotide probe 15 nucleotides in length specific for the E_β^k polypeptide. As shown in Fig. 4, this probe hybridized to a 4.8-kb *Bam*HI fragment spanning the 5′ half of the E$_\beta$ gene (see Fig. 2) in cosmids 24.2 and 8.4. This oligonucleotide probe did not hybridize to any other DNA fragment from the 200 kb of cloned *I* region. The E$_\beta$ gene also cross-hybridizes to the 5′ and 3′ probes (see below) of the human DC$_\beta$ cDNA clone. As we shall demonstrate later, this class II gene maps to the *I-A* subregion and is the site of crossing-over in the recombinant congeneic strain B10.GD as predicted from serological and biochemical analyses[34,36]. Accordingly, we believe we have correctly identified the mouse E$_\beta$ gene.

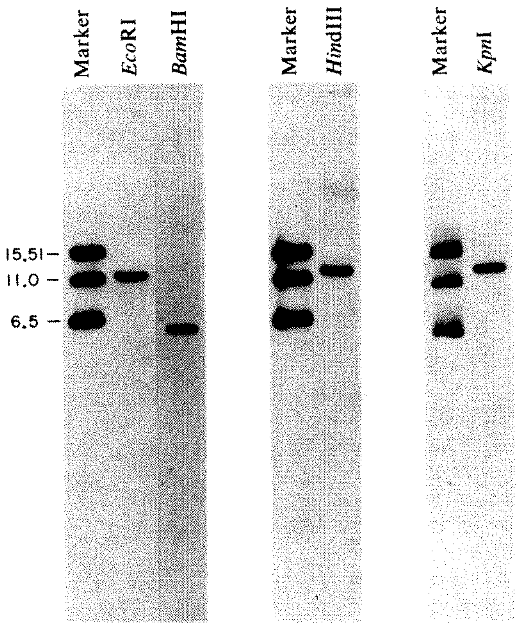

Fig. 3 Southern blot analysis of BALB/c DNA with single-copy probe 2. Ten μg of BALB/c liver DNA each was digested to completion with the enzymes indicated, separated on a 0.55% agarose gel and transferred to nitrocellulose filters. Hybridization with probe 2 (Fig. 2) was as described in Fig. 2 legend except that cold pBR322 DNA was omitted, probe concentration was 10^6 c.p.m. ml^{-1}, and the washes after hybridization were twice in 3×SSC, 0.1% SDS, and twice in 0.1×SSC, 0.1% SDS for 15 min each at room temperature. Sizes of DNA marker fragments are indicated in kilobase pairs.

A second class II *β* gene was identified between the E$_\beta$ and E$_\alpha$ genes by cross-hybridization to the human DC$_\beta$ cDNA clone. This gene hybridizes to the 3′ but not to the 5′ portion of the human DC$_\beta$ clone. The 5′ and 3′ probes from DC$_\beta$ were generated by *Pst*I digestion which cuts around amino acid

Table 1 Alleles at *H-2* regions and subregions carried by strains of independent origin and *H-2* recombinant strains

Strain	*H-2* haplotype	Parental *H-2* haplotypes	Origin of *H-2* regions *K*	*A*	*B*	*J*	*E*	*C*	*S*	*D*
A/WySn	*a*	(*k*)/(*d*)	*k*	*k*	*k*	*k*	*k* |	*d*	*d*	*d*
B10.RSF-1	*an2*	*at9/f*	*s*	*s*	.	.	*b*	(*b*)	*b* |	*f*
B10.M(11R)	*ap1*	*f/a*	*f*	*f*	*f*	*f*	*f*	*f*	*f* |	*d*
B10.S(8R)	*as1*	*a/s*	*k*	*k* |	.	. |	*s*	*s*	*s*	*s*
TBR-2	*at2*	*t1/b*	*s*	*k* |	.	. |	*b*	(*b*)	*b*	*b*
B10.RSB-1	*at9*	*t4/h4*	*s*	*s* |	.	. |	*b*	(*b*)	*b*	*b*
B10.RSB-2	*at10*	*t4/h4*	*s*	*s*	.	(*k*)	*k*	(*d*)	*d* |	*b*
B10.RSB-3	*at11*	*t3/h2*	*s*	*s*	(*s*)	(*s*)	*k*	(*k*)	*k* |	*b*
B10.RSB-4	*at19*	*t4/h4*	*s*	*s*	.	(*k*)	*k*	(*d*)	*d* |	*b*
B10.RSB-5	*at20*	*t4/pb*	*s*	*s*	.	(*k*)	*k* |	. |	*b*	*b*
C57BL/6	*b*		*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
B10.F(14R)	*bw*	*b/n*	*b*	*b*	(*b*)	(*b*)	*b* |	. |	*p*	*p*
BALB/c	*d*		*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
B10.D2	*d*		*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
B10.GD	*g2*	*d/b*	*d*	*d* |	*b*	*b*	*b*	*b*	*b*	*b*
B10.A(4R)	*h4*	*a/b*	*k*	*k* |	*b*	*b*	*b*	*b*	*b*	*b*
B10.A(3R)	*i3*	*b/a*	*b*	*b*	*b*	*b* |	*k*	*d*	*d*	*d*
B10.A(5R)	*i5*	*b/a*	*b*	*b*	*b* |	*k*	*k*	*d*	*d*	*d*
C3H	*k*		*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*
B10.P	*p*		*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*
B10.Q	*q*		*q*	*q*	*q*	*q*	*q*	*q*	*q*	*q*
A.SW	*s*		*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*
A.TL, B10.TL	*t1*	*s/a1*	*s* |	*k*	*k*	*k*	*k*	*k*	*k*	*d*
B10.HTT	*t3*	*s/t1*	*s*	*s*	*s*	*s* |	*k*	*k*	*k*	*d*
B10.S(9R)	*t4*	*s/a*	*s*	*s* |	. |	*k*	*k*	*d*	*d*	*d*
B10.RPD-1	*td1*	*t2/pb*	*p*	*p*	(*p*)	(*p*)	*p*	.	*b* |	*d*
B10.RPD-2	*td2*	*t2/pb*	*p*	*p*	(*p*)	(*p*)	*p*	.	*b* |	*d*

Vertical bars indicate presumed locations of crossing-over. Dots are given where the origin of the region is unknown. Parentheses for *H-2* alleles mean that their origin has only been deduced from the composition of the parental haplotypes. Parentheses for the parental haplotypes of strain A indicate that the *a* haplotype has not been derived from a known cross. Strains B10.RSF-1, TBR-2, B10.RSB-1, B10.RSB-2, B10.RSB-3, B10.RSB-4, B10.RSB-5, B10.F(14R), B10.RPD-1 and B10.RPD-2 were from C. David, B10.GD and B10.TL from J. Klein and C3H from the Jackson Laboratories. All other strains were raised in our colony (J.F.).

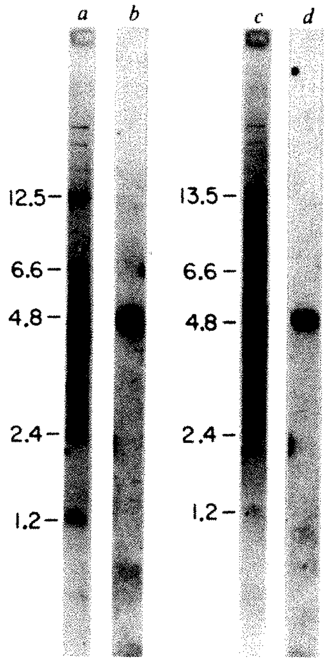

Fig. 4 Identification of the E_β gene in cosmids 8.4 and 24.2 by hybridization to a specific oligonucleotide probe. Cosmid clones 8.4 and 24.2 (0.25 μg each) were digested with *Bam*HI, separated on a 0.55% agarose gel and transferred to nitrocellulose filters. The hybridization probe, composed of a mixture of 32 15-mer oligonucleotides, was synthesized on polymer support using the phosphoramidite nucleoside chemistry[46]. The heterogeneous nucleotide sequence of the probe was based on the sequence Glu-Cys-His-Phe-Tyr (E. Sung *et al.*, in preparation) for residues 15 to 19 of the E^k_β chain. The probe was labelled with T4 polynucleotide kinase and [γ-^{32}P]ATP[47]. Hybridization was at 37 °C in the buffer described in Fig. 2 legend (except that the pBR322 and mouse DNAs were omitted) for 24 h with 5×10^5 c.p.m. ml^{-1}. The filters were washed twice for 15 min at room temperature in 3×SSC, 0.1% SDS and exposed with intensifying screens overnight. Sizes of DNA fragments are in kilobase pairs. *a* And *c* represent lanes containing ethidium bromide-stained DNA fragments from cosmids 8.4 and 24.2, respectively. *b* And *d* represent lanes containing autoradiograms of *a* and *c*, respectively, after hybridization.

residue 90 thereby separating the coding sequence for the first and second external domains[19,37]. A genomic fragment encoding the 5′ end of the E_β gene (probe 3, Fig. 2) also shows no hybridization to this second β gene. Moreover, this gene (probe 6, Fig. 2) hybridizes to the 3′ exons of the E_β gene but not detectably to the A_β gene on genomic blots in stringent conditions. Accordingly, this class II gene is E_β-like and we have denoted it $E_{\beta2}$. The 5′ exons of gene $E_{\beta2}$ might either be missing or so diversified that they no longer cross-hybridize to the corresponding mouse E_β and human DC_β probes. Thus, $E_{\beta2}$ might either be a pseudogene or another functional β gene whose product has not yet been identified by serological methods.

About 50 kb proximal to the E_β gene, a third class II β gene was identified with the human DC_β cDNA clone. As we shall see subsequently, this gene maps to the *I-A* subregion and represents the strongest band in a Southern blot analysis of BALB/c DNA with the human DC_β probe. Because this gene is distinct from the other two more closely related E_β-like genes by Southern blot analysis, it seems to be the A_β gene. Direct DNA sequence analysis has confirmed that the N-terminal amino acid sequence it encodes is identical in all positions that can be compared with the A^d_β protein sequence (M. Malissen, unpublished results).

The class II genes identified in the 200-kb region have several interesting features. First, the orientation of these genes has been determined with probes specific for the 5′ and 3′ ends of the class II genes isolated from the human DR_α and DC_β cDNAs. The E_α gene has a 5′ to 3′ orientation that is opposite of that for the A_β and E_β genes (Fig. 2). Second, there is a large intervening sequence in the E_β gene that presumably separates the exons encoding the two external domains of the β chain (see Fig. 2 legend). Third, the identification of probably three of the four genes encoding serologically defined class II polypeptides, suggests that we have already cloned a major fraction of the *I* region. Direct confirmation that these genes do indeed encode the class II polypeptides will come from gene transfer and expression studies.

The BALB/c genome seems to contain two α and four to six β genes

To estimate the number of class II α and β genes in the mouse genome, we carried out Southern blot analyses in low stringency conditions using as probes the mouse E_α gene and the human DC_β cDNA clone. These data suggest that there is a very limited number of class II genes. Figure 5*a* shows that the α probe detects the two *Eco*RI fragments of the E_α gene and a third fragment which could represent the A_α gene. Thus there appear to be only two class II α genes in the mouse genome. The human β chain cDNA probe (Fig. 5*b*) detects the two *Bam*HI fragments comprising the E_β gene and the *Bam*HI fragments containing the A_β and $E_{\beta2}$ genes. In addition to these cloned β genes, there are two or three extra bands presumably representing one to three additional β genes in the mouse genome. The human cDNA probe hybridizes most strongly to the A_β gene, suggesting that the DC_β cDNA clone represents the human homologue to the mouse A_β gene. Similar numbers of restriction fragments and presumably β genes are present in human DNA (Fig. 5*b* and C.W. *et al.*, in preparation). These data suggest that there are four to six β genes in the mouse genome. It should be stressed that there may be additional class II genes not sufficiently homologous to be detected by the α and β probes in our hybridization conditions. We can screen for these class II genes as well as for other genes located in the *I* region using the cosmid clones to examine the messenger

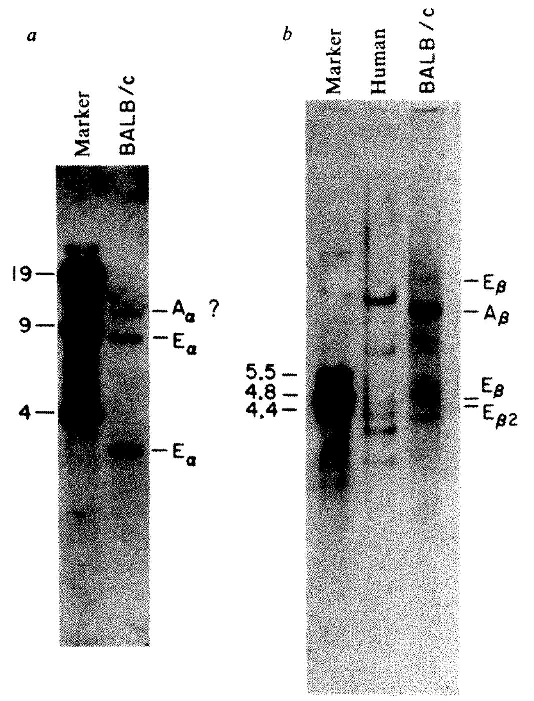

Fig. 5 Southern blot analyses of human and mouse DNAs with the mouse E_α gene and the human DC_β cDNA clone. 5–10 μg of BALB/c liver and human DNAs were digested to completion with restriction enzyme, separated on a 0.55% agarose gel, transferred to nitrocellulose filter and hybridized using the conditions described in Fig. 3 legend except that mouse DNA was omitted and the final wash was at 65 °C in 2×SSC, 0.1% SDS. Sizes of marker DNA fragments are given in kilobases. Cloned DNA fragments containing identified class II genes are indicated. *a*, BALB/c DNA was digested with *Eco*RI and hybridized with probe 7 (see Fig. 2). *b*, Human and mouse DNAs were digested with *Bam*HI and hybridized with the insert of the DC_β cDNA clone.

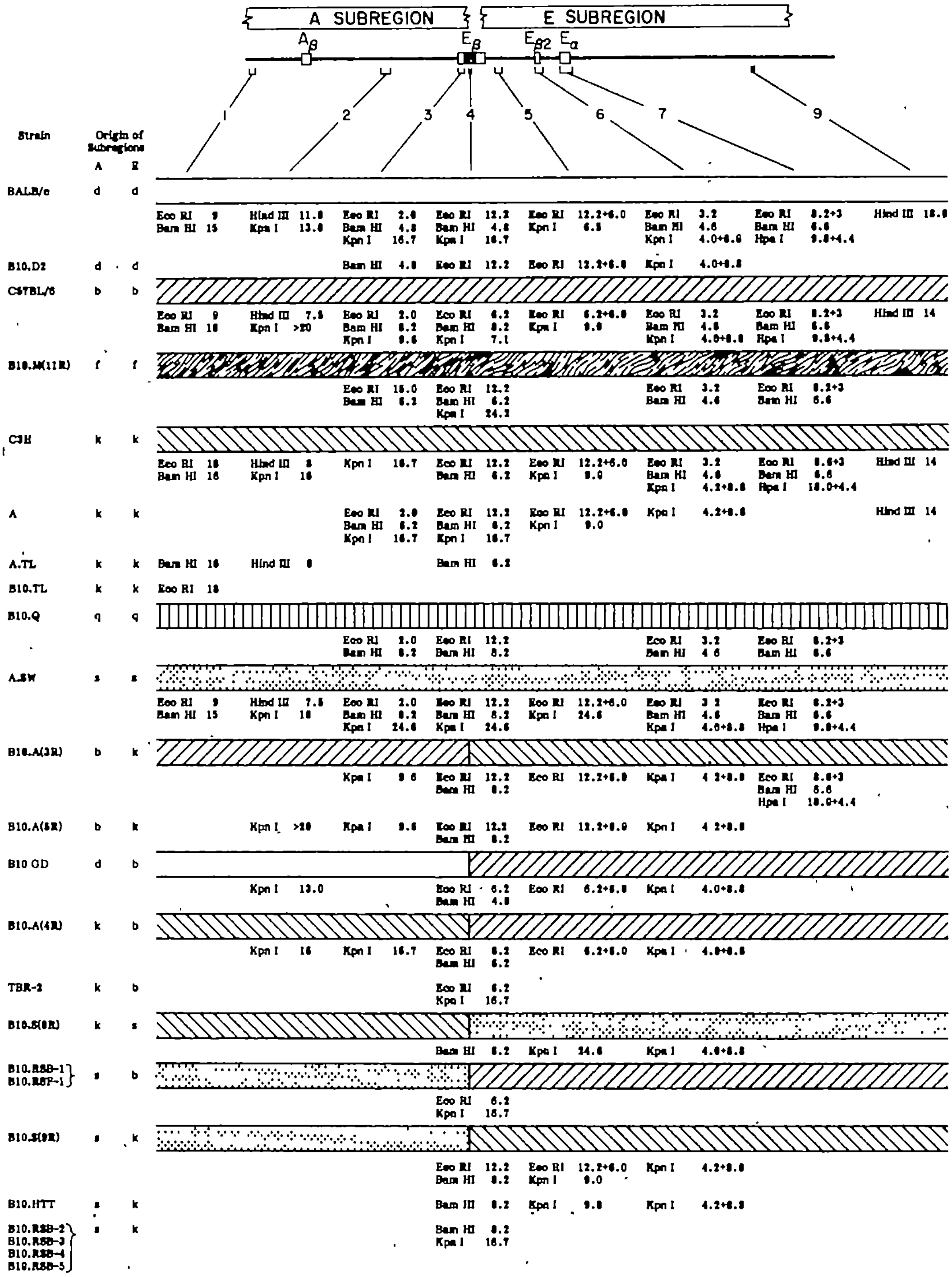
A SUBREGION
E SUBREGION
A_β
E_β
$E_{\beta2}$
E_α
1
2
3
4
5
6
7
9
Strain
Origin of Subregions
A
E
BALB/c d d
Eco RI 9 Bam HI 15 | Hind III 11.8 Kpn I 13.8 | Eco RI 2.0 Bam HI 4.8 Kpn I 16.7 | Eco RI 12.2 Bam HI 4.8 Kpn I 16.7 | Eco RI 12.2+6.0 Kpn I 6.5 | Eco RI 3.2 Bam HI 4.6 Kpn I 4.0+8.8 | Eco RI 8.2+3 Bam HI 6.6 Hpa I 9.8+4.4 | Hind III 18.0
B10.D2 d d
Bam HI 4.8 | Eco RI 12.2 | Eco RI 12.2+6.0 | Kpn I 4.0+8.8
C57BL/6 b b
Eco RI 9 Bam HI 18 | Hind III 7.5 Kpn I >20 | Eco RI 2.0 Bam HI 8.2 Kpn I 9.6 | Eco RI 6.2 Bam HI 8.2 Kpn I 7.1 | Eco RI 6.2+6.0 Kpn I 9.0 | Eco RI 3.2 Bam HI 4.6 Kpn I 4.0+8.8 | Eco RI 8.2+3 Bam HI 6.6 Hpa I 9.8+4.4 | Hind III 14
B10.M(11R) f f
Eco RI 15.0 Bam HI 6.2 | Eco RI 12.2 Bam HI 6.2 Kpn I 24.2 | Eco RI 3.2 Bam HI 4.6 | Eco RI 8.2+3 Bam HI 6.6
C3H k k
Eco RI 18 Bam HI 16 | Hind III 8 Kpn I 16 | Kpn I 16.7 | Eco RI 12.2 Bam HI 6.2 | Eco RI 12.2+6.0 Kpn I 9.0 | Eco RI 3.2 Bam HI 4.6 Kpn I 4.2+8.8 | Eco RI 8.6+3 Bam HI 6.6 Hpa I 10.0+4.4 | Hind III 14
A k k
Eco RI 2.0 Bam HI 6.2 Kpn I 16.7 | Eco RI 12.2 Bam HI 6.2 Kpn I 16.7 | Eco RI 12.2+6.0 Kpn I 9.0 | Kpn I 4.2+8.8 | Hind III 14
A.TL k k
Bam HI 16 | Hind III 8 | Bam HI 6.2
B10.TL k k
Eco RI 18
B10.Q q q
Eco RI 2.0 Bam HI 8.2 | Eco RI 12.2 Bam HI 8.2 | Eco RI 3.2 Bam HI 4.6 | Eco RI 8.2+3 Bam HI 6.6
A.SW s s
Eco RI 9 Bam HI 15 | Hind III 7.5 Kpn I 18 | Eco RI 2.0 Bam HI 8.2 Kpn I 24.6 | Eco RI 12.2 Bam HI 8.2 Kpn I 24.6 | Eco RI 12.2+6.0 Kpn I 24.6 | Eco RI 3.2 Bam HI 4.6 Kpn I 4.0+8.8 | Eco RI 8.2+3 Bam HI 6.6 Hpa I 9.8+4.4
B10.A(3R) b k
Kpn I 9.6 | Eco RI 12.2 Bam HI 8.2 | Eco RI 12.2+6.0 | Kpn I 4.2+8.8 | Eco RI 8.6+3 Bam HI 6.6 Hpa I 10.0+4.4
B10.A(5R) b k
Kpn I >20 | Kpn I 9.6 | Eco RI 12.2 Bam HI 8.2 | Eco RI 12.2+6.0 | Kpn I 4.2+8.8
B10 OD d b
Kpn I 13.0 | Eco RI 6.2 Bam HI 4.8 | Eco RI 6.2+6.0 | Kpn I 4.0+8.8
B10.A(4R) k b
Kpn I 16 | Kpn I 16.7 | Eco RI 6.2 Bam HI 6.2 | Eco RI 6.2+6.0 | Kpn I 4.0+8.8
TBR-2 k b
Eco RI 6.2 Kpn I 16.7
B10.S(8R) k s
Bam HI 8.2 | Kpn I 24.6 | Kpn I 4.0+8.8
B10.RSB-1 B10.RSP-1 s b
Eco RI 6.2 Kpn I 16.7
B10.S(9R) s k
Eco RI 12.2 Bam HI 8.2 | Eco RI 12.2+6.0 Kpn I 9.0 | Kpn I 4.2+8.8
B10.HTT s k
Bam HI 8.2 | Kpn I 9.0 | Kpn I 4.2+8.8
B10.RSB-2 B10.RSB-3 B10.RSB-4 B10.RSB-5 s k
Bam HI 8.2 Kpn I 16.7

Fig. 6 Summary of mapping data obtained by Southern blot analyses of recombinant congeneic strains and strains containing the parental haplotypes with eight different single-copy probes from the 200-kb region. The top of the figure gives the organization of the cloned 200-kb region and indicates the location of the probes used for Southern blot hybridizations. The horizontal bars marked by different symbols show the origins of the *I-A* and *I-E* subregions schematically. Vertical lines separate the *I-A* and *I-E* subregions in the recombinants. Restriction fragments detected by the probes are denoted by the enzyme used and their sizes in kilobase pairs. For probe 6 only the fragments comprising the $E_{\beta2}$ gene are listed; the fragments comprising the E_β gene detected with probe 6 due to cross-hybridization are not given. For probe 7 the weakly cross-hybridizing fragments perhaps containing the A_α gene are not listed. It should be emphasized that mouse strains of independent origin containing identical *H-2* alleles in the *I* region (BALB/c and B10.D2; C3H and A) showed no difference when analysed with our probes.

RNAs produced by B cells, T cells and macrophages. Indeed, an additional class II gene exhibiting low homology to the E_α gene has been identified between the A_β and E_β genes with B cell-specific cDNA as a probe (M. Davis, unpublished results).

The E_α and E_β genes are present in strains not expressing I–E molecules

Four inbred strains of mice of independent origin (*b*, *f*, *q*, *s* haplotypes) do not express the I-E molecule on the cell surface[38]. Two of these haplotypes (*b* and *s*) express the E_β polypeptide in the cytoplasm but fail to express the E_α polypeptide. Two others (*f* and *q* haplotypes) express neither the E_β nor the E_α polypeptides. We have used our E_α and E_β probes in Southern blot analyses to examine the DNAs of these so-called nonexpressor strains for the presence of these genes. Clearly the E_α and E_β genes are present in all nonexpressor strains (Fig. 6). Accordingly, the failure to express these class II genes is not a consequence of the deletion of the entire gene. Several different mechanisms might be responsible for the loss of expression at the transcriptional or translational levels.

Correlation of our molecular map with the genetic map of the *I* region

Immunogeneticists have divided the *I* region into five subregions by recombinational analysis of various immune responsiveness traits[6]. The various recombinant congeneic strains that we have analysed join in the *I* region two out of four independently derived haplotypes (*d*, *b*, *k* and *s*) which are represented by the inbred mouse strains BALB/c, C57BL/6, A, and A.SW, respectively. The inbred strain A (*a* haplotype) is a natural recombinant between *k* and *d* haplotype genes; since the region of interest to us in this paper in strain A is of *k* haplotype origin we will use *k* instead of *a* when we refer to the haplotype of this strain (see Table 1).

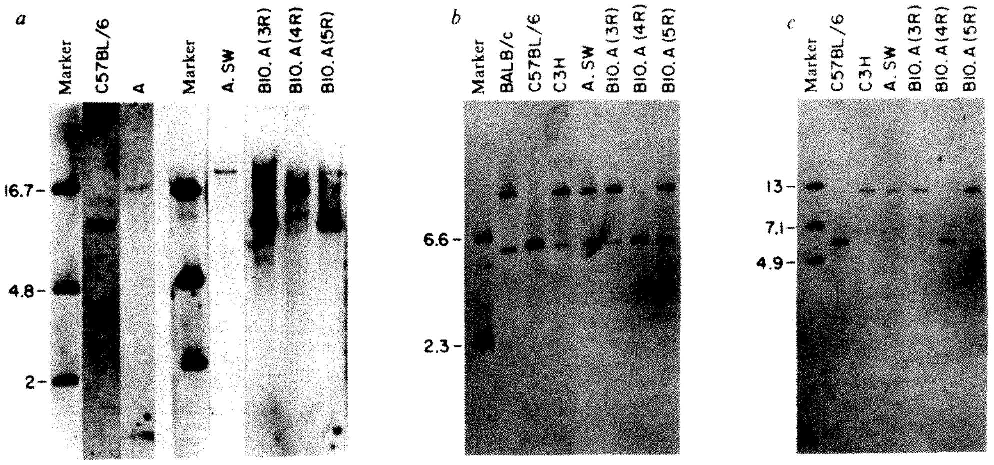

Fig. 7 Southern blot analyses of three recombinant congeneic mouse strains and of strains containing their parental haplotypes with probes 3, 4 and 5; 5–10 μg of mouse liver DNAs were digested to completion with restriction enzymes and after gel electrophoresis and transfer hybridized with single-copy probes in the conditions described for Fig. 3 except that the final washes were twice at 65 °C in 0.1 × SSC, 0.1% SDS for 15 min each. Sizes of DNA marker fragments are in kilobase pairs. *a*, Digests were done with *Kpn*I and the filters were hybridized with probe 3. *b*, *Eco*RI and probe 5. *c*, *Eco*RI and probe 4.

We correlated our molecular map with the genetic map of the *I* region through the analysis of restriction enzyme site polymorphisms as follows. First, 10 single-copy probes from various regions of the 200 kb of cloned DNA were isolated (Fig. 2). By Southern blot analyses of mouse DNA, these probes generally hybridized to single restriction enzyme fragments. Second, these single-copy probes were used to define restriction enzyme site polymorphisms between the four different haplotypes. Third, the polymorphic restriction fragments representing alternative forms of a genetic trait were used to map the probe into an appropriate *I* subregion through the analysis of a panel of recombinant strains. For example, probe 3 representing the 5′ half of the E_β gene detected 24.6-, 16.7- and 9.6-kb *Kpn*I fragments in the DNAs of A.SW (*s* haplotype), A (*k* haplotype), and C57BL/6 (*b* haplotype) mice, respectively (Fig. 7*a*). When the three intra-*I* region recombinants B10.A(3R), B10.A(4R) and B10.A(5R) were analysed with probe 3, a 9.6-kb *Kpn*I fragment was detected in 3R mice, a 16.7-kb *Kpn*I fragment was detected in 4R mice, and a 9.6-kb *Kpn*I fragment was detected in 5R mice (Fig. 7*a*). These data indicate that probe 3 is derived from a region which is of *b*-haplotype origin in 3R and 5R mice but of *k*-haplotype origin in 4R mice. Only the *I–A* subregion or the *K* region could correlate with these restriction site polymorphisms in the three recombinants (Table 1). Since probes 1 and 2 which are located proximal to probe 3 also map to the *I–A* subregion (Fig. 6), we conclude that probe 3, which encodes the 5′ half of the E_β gene, maps to the *I–A* subregion. These observations agree with the genetic map.

The surprising finding was that probe 5, which is located ~13 kb distal to probe 3, already maps into the I–E subregion although the genetic map places two additional subregions, *I–B* and *I–J*, between *I–A* and *I–E*. Figure 7*b* shows that probe 5 detects a 12.2-kb (and a 6.0-kb) *Eco*RI fragment in haplotypes *d*, *k* and *s* but a 6.2-kb (and a 6.0-kb) *Eco*RI fragment in the *b* haplotype. This polymorphism between the *b* and the *k* haplotype allows us to map probe 5 by analysis of the recombinants 3R, 4R and 5R unambiguously into the *I–E* subregion (Fig. 7*b*, Table 1). We further analyse this point below.

The finding that the two most proximal probes 1 and 2 still map into the *I–A* subregion is evident from an analysis of DNAs from B10.TL and A.TL mice and also places the A_β gene in the *I–A* subregion (Fig. 6). The $E_{\beta 2}$ gene (probe 6) and the E_α gene (probe 7) map in the *I–E* subregion (Fig. 6). With probes 8 and 9, isolated from the region distal to the E_α gene, it was more difficult to detect restriction enzyme site polymorphisms. With probe 8 we failed to detect polymorphic restriction fragments. Probe 9, which is located about 60 kb distal to the E_α gene, still maps into the *I–E* subregion (Fig. 6). In at least two recombinant congeneic strains, B10.RPD-1 and B10.RPD-2, probe 10, 25 kb distal to probe 9, maps outside the *I–E* subregion, presumably in the *I–C* subregion or the *S* region (not shown). However, in strain B10.F(14R), probe 10 still maps to the *I–E* subregion, indicating that the recombination event in 14R mice has occurred distal to the recombinational events in RPD-1 and RPD-2 mice. These results suggest that the *I–E* subregion is between 90 and 120 kb long.

The *I–B* and *I–J* subregions are confined to a 3.4-kb region of DNA

To determine more precisely the size of the chromosomal region containing the *I–B* and *I–J* subregions, we analysed nine inbred congeneic strains with recombination events between the *I–A* and *I–E* subregions. Through Southern blot analyses, we established the restriction maps for the parental haplotypes (*d*, *b*, *k* and *s*) for a region of 27 kb of DNA around the E_β gene using probes 3, 4 and 5 (Fig. 8). Polymorphic restriction enzyme sites which determine by their presence or absence in the appropriate recombinants the rightward and leftward boundaries for the recombination events are circled. The regions between these boundaries which are of undetermined origin in the recombinants, according to our present analysis, are shaded in Fig. 8.

The Southern blotting data for probes 3, 4 and 5 which led to these conclusions are summarized in Fig. 6. Several interesting points can be made from these analyses. All polymorphic restriction enzyme sites which we have analysed around the E_β gene map either to the *I–A* or *I–E* subregions (Fig. 8). No site has been mapped to the *I–B* or *I–J* subregion as defined by the appropriate recombinant strains. According to our present analysis, the maximal distance between the recombination point in B10.GD mice separating the *I–A* from the *I–B* subregion and the recombination point in B10.A(3R) separating the *I–J* from the *I–E* subregion is 3.4 kb. Figure 8 shows that the genome organization around the junction of the *I–A* and *I–E* subregions is very similar in the four mouse strains analysed which are of independent origin. Thus, one cannot postulate that a major deletion or rearrangement affecting the *I–B* and *I–J* subregions has occurred in the BALB/c mouse as compared with parental strains of the *b*, *k* or *s* haplotypes used to generate the recombinants defining the *I–B* and *I–J* subregions.

The *I–J* product may be encoded outside the *I* region

The results described above map the boundaries of the *I–A* and *I–E* subregions into a segment of DNA that is 3.4 kb. This region includes the 3′ half of the E_β gene. Indeed, if the E_β genes of the recombinant congeneic strains B10.A(3R) and B10.A(5R) are identical, as is suggested by peptide map analysis[39], the gap between the *I–A* and the *I–E* subregions may be less than 1 kb. We draw this conclusion because hybridization analysis with the human class II β probe indicates that the E_β gene extends beyond the *Hin*dIII restriction enzyme site in this region of the BALB/c genome (Fig. 8). The rightward boundary for the recombination event in B10.A(3R) and B10.A(5R) mice is marked by the polymorphic *Eco*RI restriction site 1 kb distal to this *Hin*dIII site (see Fig. 8).

This does not leave much DNA to encode the traits of the *I-B* and *I-J* regions. Indeed, the *I-B* subregion has only been defined based on the control of certain immune responses[6]. Recently, a controversy has arisen as to whether the *I-B* subregion exists in that the immune responses it controls may actually be regulated by both the I-A and I-E class II molecules[40]. Cloning studies and DNA sequence analyses of the recombinant regions defining the *I-B* subregion should allow us to localize this subregion even more precisely and, indeed, to determine whether it exists.

The *I-J* subregion apparently controls the expression of two or more serologically defined I-J gene products[41–43]. Several models may explain the gene or genes encoding I-J product(s). First, the I-J gene may be encoded outside the *I* region. This possibility cannot be excluded because the generation of the recombinant congeneic strains used to define the *I-J* subregion also involved recombination events outside the *I* region on chromosome 17. However, this possibility seems unlikely because I-J appears to segregate with the *I* region and not with any other region of the *H-2* complex. Second, expression of the I-J gene product may be controlled by a regulatory element which is encoded in this 3.4-kb region. Again this possibility seems unlikely because of the polymorphism observed in congeneic mice with identical background genes (that is, non-*I*-region genes). Third, I-J polypeptides might have been selected during ontogeny from a pool of variants for their ability to bind the E_β polypeptide in interactions between suppressor T cells and B cells or macrophages. Mouse strains polymorphic for E_β as well as recombinants affecting the E_β gene would thus select for different I-J polypeptides. The I-J polypeptide therefore seems to be under control of a locus overlapping the E_β gene, although its structural gene(s) would map elsewhere. Fourth, the I-J product may arise from RNA splicing patterns involving a polymorphic exon encoded between the *I-A* and *I-E* subregions and additional, nonpolymorphic exons encoded in the *I-A* or *I-E* subregions. This model could be analogous to the alternative RNA splicing patterns seen for the secreted and membrane forms of immunoglobulin heavy chain genes. Finally, the I-J and E_β polypeptides may be identical. The I-J chain, however, may differ in its antigenic properties from the E_β chain because of post-translational modifications such as glycosylation, or because of conformational differences if it is associated with a polypeptide different from E_α or alternatively, if it occurs as a single polypeptide chain on the surface of T suppressor cells.

Studies in progress should allow us to distinguish the first three from the latter possibilities by using a subcloned DNA segment between the *I-A* and *I-E* subregions to carry out RNA blot analyses on messenger RNAs from T-cell hybridomas expressing I-J gene products. The last two hypotheses would suggest that this region should cross-hybridize to I-J messenger RNAs, whereas the former three possibilities argue that the structural gene for the I-J polypeptide lies outside the *I* region and, accordingly, no cross-hybridization should be observed. We will also determine by cloning and sequencing, the exact location of the recombination points in, for example, B10.A(3R) and B10.A(5R) mice which define the leftward and rightward borders of the *I-J* subregion. Indeed, it is possible that the *I-A* and *I-E* subregions will be contiguous with no intervening DNA that could encode the *I-B* or *I-J* subregions. If so, the *I-B* and *I-J* subregions cannot be located between the *I-A* and *I-E* subregions.

Recombination in the *I* region is sharply localized in and around the E_β gene

The recombination events which occurred in nine independently generated recombinant congeneic mice between the *I-A* and *I-E* subregions have been mapped (Fig. 8). These recombination points all map near to the E_β gene within a 9.8-kb segment of DNA. Indeed, serological and peptide map analyses suggest that the recombinational event in B10.GD mice occurred in the E_β gene[34,36]. This result is in agreement with our chromosomal mapping studies using restriction enzyme site polymorphisms. We carried out several types of control experiments. First, in certain cases we analysed doubly recombinant strains of mice which had been generated by genetic crosses between one of the above recombinant congeneics and a new parental strain. In each case where the *I*-region recombinant haplotype was retained, the same polymorphic restriction fragments were observed as in the original parental strains (Table 1, Fig. 6). Second, critical strains B10.A(3R), B10.A(5R), B10.S(8R), B10.S(9R) and B10.HTT were obtained from other laboratories (D. Murphy and C. David) and gave the same results as the identical strains raised in our colony (J.F.). From a comparison of the lengths of those restriction fragments (Fig. 6) which span the recombination points in the appropriate recombinants with the maps of the parental haplotypes in Fig. 8, it is clear that the recombination events have occurred without any major duplication or deletion of DNA sequences. For example, in Fig. 7*c*, a 12.2-kb *Eco*RI fragment is detected in B10.A(3R) and B10.A(5R) and a 6.2-kb fragment is found in B10.A(4R) with probe 4 which is derived from the large intervening sequence in the E_β gene. As can be seen from Fig. 8, these *Eco*RI fragments span the recombination points in the recombinants. The sizes of these *Eco*RI fragments support the

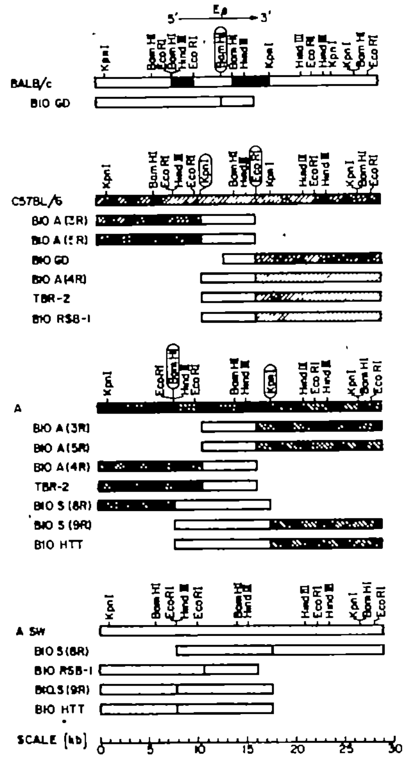

Fig. 8 Partial restriction maps for 27 kb of DNA around the E_β gene in mouse strains of *d*, *b*, *k* and *s* haplotypes. These maps indicate the regions where crossing-over occurred in nine independently generated recombinant congeneic strains. The restriction maps for the four mouse strains were obtained by Southern blot analyses of single and double digestions of mouse liver DNAs with the indicated enzymes using probes 3, 4 and 5 as hybridization probes (see Fig. 2 for the location of these probes). Compared with Fig. 2, the map for BALB/c shows only the restriction sites mapped by Southern blotting. Maps obtained by Southern blotting might not reveal all sites for the enzymes used since sites outside the fragments detected with the hybridization probes are not seen. This limitation of the method, however, does not affect any of our present conclusions. The location and maximal size of the 5' and 3' exons of the E_β gene are indicated in the BALB/c map by black boxes (see Fig. 2 legend for a discussion of the organization of this gene). Those regions of the four chromosomal DNA segments that are present in the nine recombinant congeneic mouse strains according to our analyses, are indicated by appropriately marked bars below the restriction maps. The grey boxes indicate the regions in the recombinants where the crossing-over has occurred and which cannot be assigned at present to either parent because of the lack of polymorphic restriction sites. Polymorphic restriction sites confining the regions of recombination are circled.

hypothesis that the recombinants have been generated by a homologous crossing-over event.

The recombinational events in the nine independently generated recombinants analysed could all have occurred at precisely the same site. As discussed above, we are in the process of subcloning several of these recombinant regions to identify in molecular terms their precise points of recombination. These observations suggest that in the *I* region, recombinational events are not random but rather occur in certain highly localized regions. This obviously has important implications for any attempts to correlate genetic maps that are determined by recombinational analyses and molecular maps determined by direct cloning. For example, the A_β and the E_α genes are by recombinational analyses about 0.1 centimorgan apart[2]. As the mouse genome is 1,600 centimorgans long and contains about 3×10^6 kilobars pairs, 0.1 centimorgan corresponds to about 200 kb (if on the average recombination is random throughout the mouse genome). We have now established directly that the A_β and E_α genes are separated by about 85 kb of DNA (Fig. 2) and that all the recombinational events analysed in this region occurred within 9.8 kb of DNA. One wonders if genetic maps for other regions of the eukaryotic genome will show similar recombinational preferences. In particular, it will be interesting to determine whether there are other hot-spots of recombination within the major histocompatibility complex, as for example between the *I–E* subregion and *S* region or between the *S* and *D* regions—two sites where many cross-overs have been mapped.

The future

The cloning of the *I* region will permit the precise identification of genes involved in immune responsiveness and other phenotypic traits encoded by this region. We will be able to use the techniques of gene transfer and *in vitro* mutagenesis to determine which genes control specific traits and how they function to regulate these traits. The cosmid clones encompassing the *I* region can be used to determine whether other previously undetected *I*-region genes are expressed in cells that control various aspects of the immune response such as B cells, T cells or macrophages. The detailed knowledge of the *I–A* and *I–E* loci will be of great value in the analysis of the human *I* region at the molecular level. Finally, it is clear that the genetic map of the *I* region can now start to be replaced with a molecular map which localizes precisely the genes encoding the various types of class II polypeptides.

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LETTERS TO NATURE

Convective cloud merging and its effect on rainfall

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An important aspect in the study of cumulonimbus convection is the identification of situations that lead to intense rainfall. We examine here one such situation in which two initially separate clouds interact and merge. The process is found to depend on the variation of wind with height, the relative stage of development of the two clouds and their initial separation. A numerical simulation is described in which merging is shown to increase the total surface precipitation by a factor of four.

Simpson *et al.*[1] investigated the development of merging cumulus when the two separate clouds were initially two or three cloud diameters apart. They suggested that new growth could take place above colliding gust fronts formed by the downdraughts from each cloud. Large accumulations of rainfall resulted from the increased size and duration of the cloud complex. Here an alternative mechanism of merging is considered in which the amount of rain is increased by enhancing the rainfall rate[2]. The work is a development of an idea discussed by Orville *et al.*[3] in which two separate cumulonimbus clouds grow in close proximity, closer than that necessary for the merging process described by Simpson *et al.* Under favourable combinations of vertical wind shear, cell separation and relative cloud development, the cloud motion fields (as opposed to the downdraughts) interact and the clouds merge to form a single entity.

The effects of this type of merging should be evident in the rainfall characteristics of a field of convective clouds, particularly if those clouds are similar in size and growing in uniform conditions. On such occasions most clouds might be expected to have similar rainfall, but a few, growing sufficiently close, would merge, the consequent enhancement in rainfall rate being dependent on the effectiveness of the merging. Such rainfall characteristics have been observed using a weather radar[4,5]. The peak rainfall rate of most of the clouds fell within

Table 1 A comparison of the water budgets of the single cell and merged clouds

		Single cell	Merger	Difference merger − single
1	Total available condensed cloud substance CN	3.8×10^8 kg	10.12×10^8 kg	+33%
			(Fractions per unit CN)	
2	Total precipitation generated within the cloud comprising of	0.55	0.61	+0.064 (+12%)
	a, accretion of cloud water by rain and hail	0.23	0.31	+0.080 (+35%)
	b, other processes (such as autoconversion of cloud water to rain, sublimation of cloud ice to graupel)	0.32	0.30	−0.016 (−5%)
3	Precipitation evaporated below cloud base	0.47	0.39	−0.086 (−18%)
4	Net precipitation reaching the ground as rainfall	0.07	0.22	+0.15

Water budgets: 1, The net condensed cloud substance (CN): the percentage change in the third column is calculated using CN from two single cells. 2, The total amount of precipitation produced within the cloud together with a breakdown of the physical processes involved. 3, The evaporation. 4, The surface rainfall, that is the total amount produced minus that evaporated. All values in 2, 3 and 4 are expressed as fractions of CN.

the range 5–15 mm h^{-1}, but up to 30% had a much higher rainfall rate, in the range 30–100 mm h^{-1}. It has been suggested that the second group might have resulted from merging. To investigate this, both single and merging clouds have been simulated with a tuned three-dimensional numerical model[6] using representative thermodynamic and dynamic data[5]. Details of the microphysical and turbulent parametrization schemes, together with the model boundary conditions, are given in ref. 6, but for brevity are omitted here.

Figure 1 shows the early development of two clouds, separated in distance by 7 km and in time of initiation by 6 min. Each shows a vertical section through the centre of the cloud parallel to the direction of the mean wind. At a simulated cloud time of 12 min the motion field of the larger cloud has not developed sufficiently to influence the growth of the other and both clouds lean to the right, as would be expected in the prevailing conditions. By 16 min the clouds have merged perceptibly. Large ice particles begin to form in the more mature cloud and, by 20 min, their concentration has reached 0.05 g per kg and their fall speed, in still air, is ~6 m s^{-1} (ref. 6). At this stage they can be considered as 'embryo precipitation particles'.

The instantaneous velocity vectors are evaluated relative to the mean speed of the larger cloud which corresponds to the wind at 725 mbar (~3 km above the surface) in the undisturbed air. Relative to this frame of reference the inflow and outflow are clearly evident. The clouds move at slightly different speeds because of their different size (which affects their interaction with the undisturbed flow), and their mutual interaction. As a result, the region of high liquid water content (>2 g per kg) in the lower part of the right hand cloud is advected towards the left (relative to the frame of reference defined above), and by 24 min (Fig. 1*d*), is positioned so that subsequently the precipitation falls through it, the growth of the precipitation thereby being greatly enhanced by accretion of numerous small water droplets. This process, whereby precipitation generated in one cloud is augmented as it passes through another, is similar to that encountered near high ground[7] where precipitation generated from frontal cloud grows by accretion of orographically induced cloud which forms lower down, just above the hill.

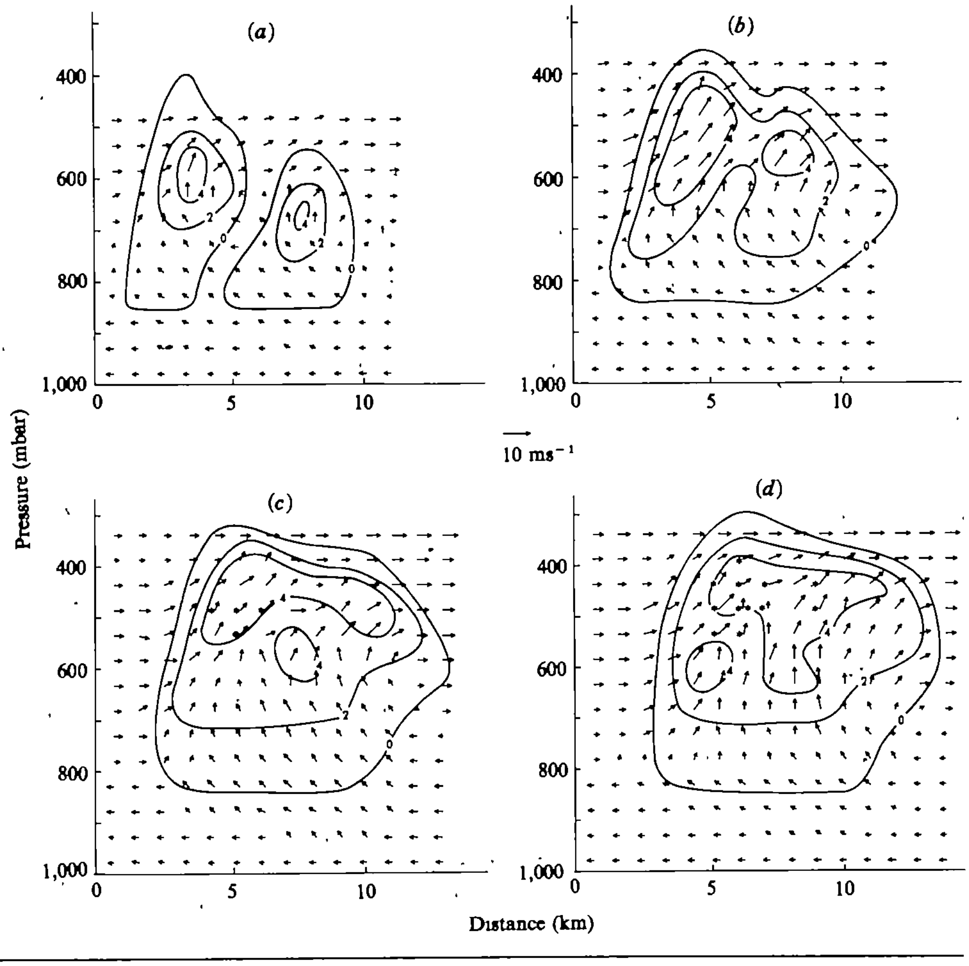

Fig. 1 Distribution of cloud content (water and ice) in g per kg within the clouds at a simulated time of: *a*, 12 min; *b*, 16 min; *c*, 20 min; *d*, 24 min. Arrows denote wind velocities in the plane of the diagram relative to the mean speed of the left hand cloud. The presence of rain (•) and hail (*) is also indicated.

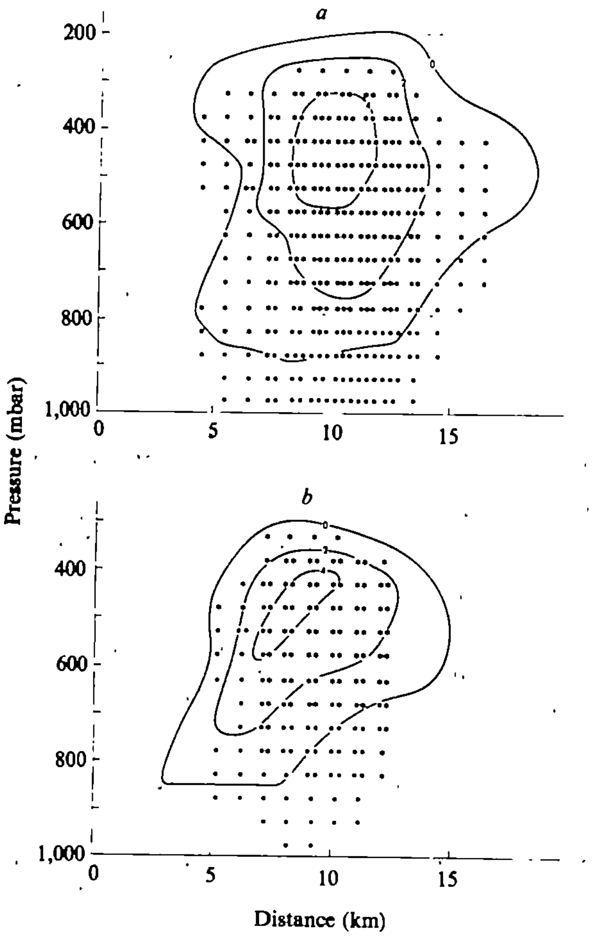

Fig. 2 Distribution of rain (•), hail (*) and cloud substance (water and ice) at a simulated time of 36 min within: *a*, the merged cloud; *b*, the single cell. Isopleths of cloud content are in g per kg. The number of symbols in each group represents concentrations of <0.5, 0.5–2.0 and >2.0 g per kg. These approximate to rainfall rates of <8, 8–44 and >44 mm h^{-1} respectively.

The second region of high liquid water content low down on the left hand side of the cloud in Fig. 1*d* cannot influence the growth of the precipitation by accretion because it is in an unfavourable position. During the subsequent development it rises to the top of the cloud to provide a continuing supply of precipitation embryos. A later stage in the development when merging is complete is shown in Fig. 2*a*. There is a high concentration of rain and hail in the centre of the cloud and the maximum modelled instantaneous rainfall rate at the surface (1,000 mbar) is ~100 mm h^{-1}. Comparison between this and a modelled single cell cloud (Fig. 2*b*) growing in the same environment shows that the merged cloud produces ~4 times more total surface precipitation than two individual clouds growing separately, and there is a 10-fold increase in the maximum instantaneous surface rainfall rate. (Both the merged and single clouds have a similar size and structure in the cross-shear plane.)

The rainfall characteristics of both simulated clouds are shown in Fig. 3 together with observations[5] from radar. The observed rainfall rates, averaged over 4 km^2, are the highest within the radar echo of the cloud; the modelled rates are the means of the four grid values (each 1 km^2) centred on the simulated peak surface rainfall. Both sets of values are subject to some uncertainty. Calibrated radar observations are generally considered to be representative to within a factor of ~1.5 but it is difficult to be as precise in estimating the errors in the numerical model. There are uncertainties in the parametric representation of the microphysical and turbulent diffusion processes as well as in the effects of the lateral boundary conditions. An attempt has been made to 'tune' the model[6] to reduce these uncertainties but it would be unwise to suggest that the predictions had an accuracy greater than a factor of 2, although a direct comparison between simulations has the merit that many of the uncertainties at least remain constant. In consequence, the agreement between observed and modelled peak rates is fortuitously close, but there is no doubt about the difference in rainfall rates between the single cell and merger clouds.

Comparison between the two simulations shows that more rain reaches the ground from the merger because more precipitation is produced by accretion within the cloud and there is less evaporation below cloud base. The relevant quantities are shown in Table 1. The production of precipitation by accretion and loss by evaporation are expressed as fractions of the mass of cloud substance (water and ice) available for the production of precipitation. This quantity (CN) represents the difference between the cloud substance condensed and that evaporated at the cloud boundary.

The total precipitation produced per unit CN within the merged cloud is 12% greater than that within the single cell because a higher proportion of cloud water is accreted in a merger. The proportion of precipitation generated by other microphysical processes is similar in both clouds; in fact, there is slightly less in a merger because the cloud water content has been depleted by accretion. In addition, the amount of precipitation lost by evaporation below a merger is 18% less than that below a single cell. This results in the fraction of precipitation reaching the ground from the merger being three times greater than that from the single cell. However, CN in the merger is more than twice that in the single cell and, expressed in absolute terms, the mass of precipitation reaching the ground from the merged cloud is four times that from two single cells.

The increased efficiency of precipitation production within the merger can partly be explained by its increased size. The relevant parameter is the horizontal displacement of a precipitation particle as it falls compared with the 'width' of the cloud. This determines the proportion of time spent by the particle accreting water inside the cloud to that spent evaporating outside it. The efficiency also depends on the dynamic interaction of the clouds which determines the relative position of the precipitation and regions of high liquid water content.

At present the relative importance of cloud size and the dynamical effects of merging are not known. However, supple-

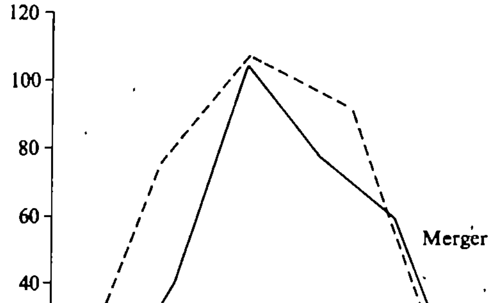

Fig. 3 Simulated and observed rainfall rates for merged and single cell clouds. The dashed curve labelled 'single' depicts observed rainfall rates that were typical of the smaller clouds and the dashed curve labelled 'merger' the rainfall rates of the largest cloud observed[5]. The largest was chosen as the numerical simulation (solid line) shows the results of the most efficient merger achieved. Note that, in the text, comparisons are between mergers and two single cell clouds. The simulation shown here labelled 'single' (solid line) represents one single cell cloud to enable direct comparison with observations.

mentary numerical simulations have shown that merging can both increase the surface rainfall by a factor of four, and decrease is by ~40%. A reduction in rainfall can occur when the wind field of the primary cloud dominates that of the secondary which, in the limit, is drawn into the updraught of the primary cloud before it has grown past its embryonic stage. Consequently the large variability often observed in convective precipitation[5] can be accounted for even without considering the natural environmental variability that is almost certainly present within any air mass.

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Cyclic thermal transformations in caesium graphite

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Various physical properties can yield novel information about effects of texture of a graphite, on the detailed course of any thermal transformation of a graphite intercalate, when it is subjected to cyclic changes of temperature including a lambda peak. Using a DSC calorimeter with an imposed temperature change of 10 K min^{-1}, we have found a novel transformation in caesium graphite CsC_8 around 250 K. We show here that fine details appear to be sensitive to the texture of the graphite used.

When electron donor or electron acceptor molecules intercalate into graphite, this pushes the carbon hexagon sheets apart by several nanometres. Any occasional defect-bonding between neighbouring parallel carbon hexagon sheets becomes stressed by this enforced separation, and in near-ideal ordered graphites 'rogue bonds' between the sheets may even snap. On lessening this stress by removing the intercalated molecules, in favourable cases the rogue bonds may not reform. Planned sequences of intercalation and removal might thus in principle be made a means for permanently improving the quality of well ordered graphites. Unfortunately, the graphites and pyrographites available in the early experiments were not sufficiently well ordered to test sequences of intercalation and removal adequately. It was found that a proportion of the intercalated molecules remained 'locked-in', as so called 'residue compounds'[1]. Use of better oriented graphites, as prepared by subjecting pyrographites to stress annealing[2–4], gives a less confused response to sequences of intercalation and removal of the intercalated molecules, particularly when optimum behaviour of the lamellar framework is attained, by careful quality selection[5] of highly oriented pyrolytic graphite (HOPG).

When a graphite intercalate undergoes one or more thermal transformations on traversing a range of temperatures, even more gentle stresses can be imposed through cyclic changes of temperature; for example, there are changes of structure but no changes of composition in cycling the temperature around a lambda point. With this in view, various thermal transformations in graphite intercalates are being studied. These present some novel features in the chemical physics of solids, where previous research has been largely restricted to three-dimensional structures. Quite often a choice can be made between several different physical properties for study in relation to the thermal transformation. For example, the lambda transformation in graphite nitrates and deuterates with a peak temperature around 252 K has been followed in cyclic temperature changes using observations of changes in thermal expansion, lattice structure, electrical resistivity, thermal e.m.f., and pulsed NMR[6–9]. Because of the extremely high anisotropy of these compounds, as the temperature is varied the sharpness of peak change in any one layer may differ notably from the (often much lessened) sharpness of the correlation between neighbouring layers. In the limit, each layer of intercalate might be effectively independent of even the nearest neighbour layers; effectively two-dimensional thermal transformations would be of considerable theoretical importance as well as practical interest. But in practice, at least weak correlation of molecular behaviour can generally be observed between neighbouring layers, up to (say) five away. These considerations make it desirable whenever feasible to choose physical properties which can be conveniently studied parallel and perpendicular to the layers, independently. For example, to enlarge the possibilities, methods of measuring resistivity changes perpendicular to the layers have recently been improved[5].

Knowledge of the enthalpy and entropy changes provides important background information for interpreting any of the other physical measurements on transformations in intercalation compounds. A 'rate of enthalpy change' calorimeter has recently become available which has the advantage of requiring only a few milligrammes of substance. Despite certain limitations, such as the rather fast changes of temperature that must be used, this calorimeter has given interesting preliminary information about graphite nitrates[10]. It has now been used with caesium graphites. Both the first stage and second stage intercalates have been studied, but for simplicity the present report deals principally with the first stage intercalate CsC_8. Unexpectedly, calorimetry reveals a thermal transformation in the temperature range around 250 K, which has not been hitherto detected either in resistivity studies, or detailed X-ray diffraction measurements[9] in CsC_8.

Our general methods followed regular two-bulb preparative techniques. To permit comparisons between graphites of different textures each preparation exposed four different specimens of ~25 mg side by side to the caesium vapour, in conditions appropriate for obtaining the first or the second stage intercalate. After completion of intercalation the specimens were covered with non-reactive petroleum jelly[11]. It was verified that *per se* this film of jelly (which facilitates handling particularly after intercalation) makes a negligible contribution to the calorimetric anomalies now reported: interestingly the jelly showed a very weak glass-type transformation at considerably

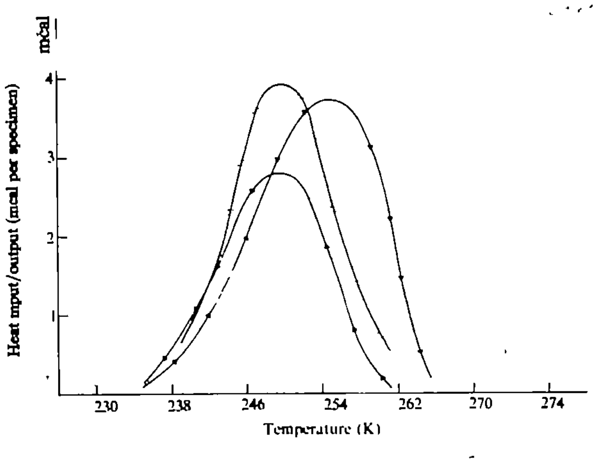

Fig. 1 Heat input/output for two different specimens of CsC_8. For HOPG (▼) the direction of temperature changes imposed follows the arrows. For Ticonderoga (natural) graphite (☐) only the cooling curve is shown.

higher temperatures, but this could not be shown on the scale used for Fig. 1.

Following earlier indications of its importance (see ref. 12) in the present studies particular attention was paid to the textural quality of the graphites used for intercalation. Two graphites of different origins were used. Figure 1 refers to stress-annealed pyrographite carefully selected by us from samples of HOPG (provided by A. Moore of Union Carbide), and to a piece of Ticonderoga graphite broken off from a fairly large specimen of this geological deposit. As anticipated, the thermal behaviour of CsC_8 although prepared under identical vapour pressure of caesium differed in fine detail, depending on which graphite was intercalated, and whether the temperature was rising or falling through the transformation, roughly between 238 and 262 K (Fig. 1). The origin of rate dependent differences between the two samples cannot yet be pinned down with certainty. Rate-controlled input or abstraction of heat is used with the present type of calorimeter; so far, only heat transfers accompanying temperature changes of 10 K min^{-1} have been used exhaustively. With such a simple type of intercalate as atoms of caesium, many solid state processes should be too rapid to be distorted by the rates of change of temperature imposed on the calorimeter. Provisionally, the findings suggest some kind of order/disorder, with details controlled by the texture of the carbon-hexagon layers in the two varieties of graphite studied; suggestions have been made with reference to transformations in CsC_{24} (ref. 9) which appear to be related to the thermal transformations now reported in CsC_8.

The magnitude of the thermal transformation is also noteworthy. Approximate integration of the calorimetric data indicates a total enthalpy change of about 26.7 cal per g atom, with an entropy change of only about 0.1 e.u.

Observations that the texture of the graphite can have such marked influence on the fine detail of thermal transformations of intercalates emphasize the need for graphites of better quality than any yet available, when seeking to establish unusual two-dimensional effects such as phonon–electron interactions and superconducting transitions.

Note that although the choice of intercalates with such strong electron donor character as caesium for the present research has been made because they present a special theoretical interest, they also enhance the practical difficulty that these intercalate atoms are highly reactive chemically. Our experience with this novel calorimeter is limited. Despite all the precautions taken against the intrusion of reactive species other than graphite, and caesium, part of the interesting non-reversibility which may be seen in Fig. 1, could be due to some permanent loss of caesium in each cycle, by combination with other intruder molecules. However, a dominant role of the texture of the graphite, in determining fine differences between different specimens, seems certain.

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Pu, ^{241}Am and ^{137}Cs in soil in West Cumbria and a maritime effect

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Discharges to the Irish Sea from Sellafield (formerly Windscale) in Cumbria in the period 1957–79, included about 16,000 Ci of Pu α activity and 800,000 Ci of ^{137}Cs, in addition to other actinides and fission products[1–6]. Most of the ^{137}Cs in the sea remains in solution whereas ~95 % of the Pu is removed to sediments[7]. To ascertain whether any of this discharged material was being transferred back to land, samples of soil were taken along two transects normal to the Cumbrian coast and extending up to 20 km inland. We report here that Pu and Am deposits decrease with distance inland and correlate with deposits of marine-derived sodium. An enrichment of actinides in seaspray relative to seawater is required to account for the observed deposits.

Samples from the first transect, between St Bees and Ennerdale, were collected in May 1977 and from the second, between Nethertown and Newlands, in July 1979. The locations of these transects and the individual sampling sites are shown in Fig. 1. Soil was taken from apparently undisturbed sites to a depth of 15 cm using core tubes of 3.8-cm diameter. Three cores were taken at each location, combined, dried at 105 °C, ground to a particle size of $< 150\ \mu m$ and homogenized. Approximately 100 g of each bulked sample were taken for analysis by γ-ray spectrometry, using lithium drifted germanium detectors. ^{137}Cs was determined from the spectra by least squares fitting using automatic data processing[9,10]. Plutonium was determined on 70 g samples by an acid leaching technique[11], using ^{242}Pu as an internal tracer. The amounts of ^{238}Pu and $^{239+240}$Pu in electrodeposited sources were determined by α spectrometry. Soils from the Nethertown transect were also analysed for ^{241}Am by radiochemistry and α spectrometry, using ^{243}Am as a tracer. The analytical results are presented in Table 1, radionuclide concentrations being expressed as deposits per cm^2 to a depth of 15 cm.

To evaluate the results, the deposit of radionuclides in soil due to fallout from distant nuclear weapons must be considered. The accumulated deposit depends mainly on rainfall[12] and latitude[13]. Results from a nationwide survey[14] have shown that, in Britain, the ^{137}Cs deposit (to a depth of 15 cm) is ~ 7.5 pCi cm^{-2} per m of rain. The $^{239+240}$Pu deposit is ~ 0.14 pCi cm^{-2} and that of ^{238}Pu, including a contribution from a satellite burnup[15] in 1964, is ~0.006 pCi cm^{-2}. The fallout baseline for ^{241}Am is not so well established. However, the mean ratio of ^{241}Am to $^{239+240}$Pu on the Nethertown transect between 10.9 and 20.5 km inland is 0.23, a value which agrees with the global estimate of Krey *et al.*[16]. This ratio suggests a ^{241}Am deposit from the testing of nuclear weapons of ~ 0.032 pCi cm^{-2} per m of rain. The weapons fallout contribution at each site has been estimated from the mean annual rainfall

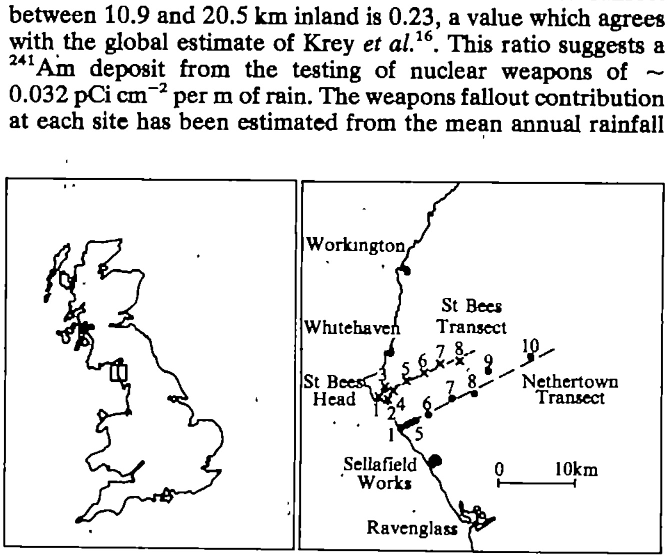

Fig. 1 Location of soil sampling sites.

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Table 1 Deposits of ^{137}Cs, ^{238}Pu, $^{239+240}$Pu and ^{241}Am in soil in West Cumbria

	St Bees to Ennerdale (1977)					Nethertown to Newlands (1979)					
Site no.	Distance from sea (km)	Estimated rainfall (mm yr^{-1})	^{137}Cs	^{238}Pu	$^{239+240}$Pu	Distance from sea (km)	Estimated rainfall (mm yr^{-1})	^{137}Cs	^{238}Pu	$^{239+240}$Pu	^{241}Am
1	0.02	975	9	0.19	1.38	0.05	1,000	11.0	0.22	1.56	1.30
2	0.25	990	11	0.09	0.72	0.20	1,000	10.2	0.12	1.03	0.80
3	2.0	1,100	15	0.03	0.48	0.50	1,000	8.9	0.10	0.96	0.56
4	2.5	1,140	12	0.04	0.68	0.90	1,000	7.4	0.05	0.81	0.40
5	7.0	1,430	16	0.02	0.41	1.60	1,050	11	0.04	0.59	0.21
6	10.0	1,630	13	0.02	0.31	5.1	1,450	12	0.04	0.47	0.14
7	12.5	1,800	16	<0.01	0.18	8.0	1,800	15	0.02	0.61	0.11
8	13.75	1,800	15	0.02	0.29	10.9	2,000	9.6	0.03	0.51	0.10
9	—	—	—	—	—	14.3	2,350	25	0.02	0.51	0.13
10	—	—	—	—	—	20.5	2,130	19	0.02	0.41	0.10

Values are pCi cm^{-2} to a depth of 15 cm.
The uncertainties, $2\sigma_\alpha$, on the above results associated with counting statistics are ~30%, 25%, 10% and 15% for ^{137}Cs, ^{238}Pu, $^{239+240}$Pu and ^{241}Am respectively. The variability of sampling, $2\sigma_s$, is generally <25%.

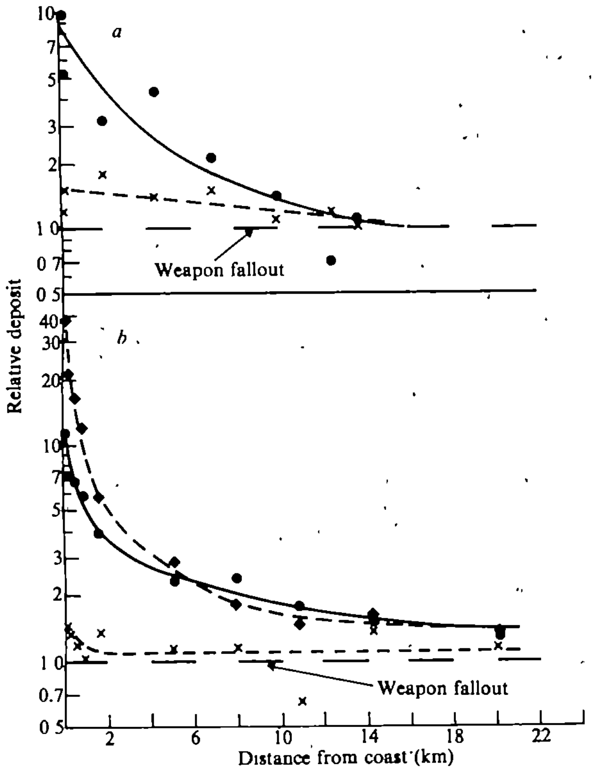

Fig. 2 Deposits of ^{137}Cs(×), $^{239+240}$Pu (●) and ^{241}Am (◆) in soil, relative to fallout from nuclear weapons tests. *a*, St Bees Transect (1977); *b*, Nethertown Transect (1979).

using data published by the Meteorological Office[17]. In Fig. 2*a* the deposits of ^{137}Cs and $^{239+240}$Pu at each site on the St Bees transect have been plotted, as a ratio to the estimated deposit due to fallout, against distance inland. Figure 2*b* shows similar results for the Nethertown transect, including data for ^{241}Am.

The $^{239+240}$Pu deposit at the coast on both transects is about a factor of 10 greater than expected from nuclear weapons fallout. As may be seen from Table 1, the ratio ^{238}Pu/$^{239+240}$Pu falls with increasing distance from the coast. At the coast, the value of this ratio is characteristic of the highly irradiated material discharged from Sellafield[5,6]. The relative deposit of ^{241}Am near the coast on the Nethertown transect is greater than that of $^{239+240}$Pu but it decreases in a similar way with distance inland. In contrast, the deposit of ^{137}Cs in both transects is only slightly enhanced at the coast and rapidly becomes indistinguishable from nuclear weapons fallout.

Figure 3 shows the excess deposits of ^{238}Pu and $^{239+240}$Pu on the St Bees transect plotted against distance from the coast. Included in Fig. 3 are values for the annual sodium deposit derived from measurements at several sites in the United Kingdom[18,19] and in Holland[19]. The latter agree with data reported elsewhere[20]. It is apparent that the slopes of the three lines are broadly similar, demonstrating a correlation between Na and Pu deposition. This, together with the general shape of the deposition curves, is taken as good evidence that the excess Pu is of maritime origin and associated with seaspray. A similar maritime effect has been reported[21] in the coastal region of Normandy, France, near the fuel reprocessing works at La Hague.

The excess deposit of $^{239+240}$Pu in soil at St Bees is 1.2 pCi cm^{-2} and the annual Na deposit at the coast (10^4 μg cm^{-2} from Fig. 3) is equivalent to $\sim 10^{-3}$ l cm^{-2} of seawater. Approximately 70% of the Pu discharged to sea between 1957 and 1977 was discharged after 1970, when the concentration of $^{239+240}$Pu in filtered inshore seawater was reported[7] to be ~0.5 pCi l^{-1}. The expected annual deposit of $^{239+240}$Pu from seaspray at the coast would therefore be ~ 5×10^{-4} pCi cm^{-2}, or 3×10^{-3} pCi cm^{-2} over a 6 yr period. An enrichment factor of 2 or 3 orders of magnitude in seaspray is therefore required to account for the excess $^{239+240}$Pu in soil along the St Bees transect. An analysis of ^{241}Am deposits in the Nethertown transect indicates that a similar enrichment factor is required to account for the deposition of this radionuclide. However, the deposition of ^{137}Cs in both transects can be accounted for by levels in bulk seawater with no evidence of enrichment.

Possible mechanisms which may be involved in the transfer of radioactivity from sea to land are: (1) direct suspension of the sea surface by the wind in the form of spray; (2) conversion of the sea surface material into aerosol by bubble bursting[22,23];

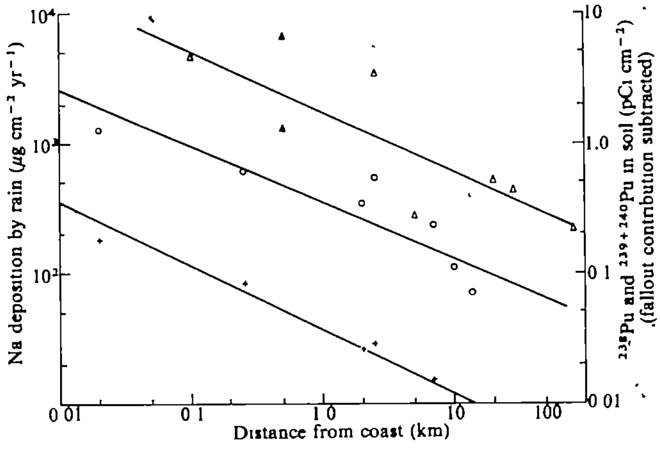

Fig. 3 Deposition of Na(△), ^{238}Pu (+) and $^{239+240}$Pu (○) versus distance from the sea (1977).

(3) injection of seawater and suspended sediment into the air by waves breaking in the surf zone; and (4) movement of sediment to intertidal regions with subsequent resuspension by wind. Of themselves, the results do not favour any one of the above mechanisms but the apparent existence of an enrichment effect for Pu and Am in seaspray, and its absence for ^{137}Cs, suggests that the mechanism may be related to the sediment in seawater rather than to material in solution. Perhaps the most likely mechanism is the production of high concentrations of suspended particulates in the surf zone produced by the action of breaking waves and the consequent injection of particulates into the atmosphere with spray.

To put the observed values in perspective in relation to hazards they can be compared with some proposed limiting values. The highest observed deposit of Pu was ~2 pCi cm^{-2} in a 15 cm depth of soil. This is more than a factor of 1,000 below the Generalized Derived Limit for Pu in well-mixed soil as specified by the National Radiological Protection Board[24].

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CH_4, H_2, CO and N_2O in submarine hydrothermal vent waters

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Hydrothermal circulation systems of mid-ocean ridges profoundly influence the chemistry of the oceans and the oceanic crust[1–3]. This has been demonstrated for several major and minor constituents of seawater[4] and trace metals[5]. In addition, several volatile compounds including helium[6,7] as well as methane and hydrogen[8–11] are introduced to the sea floor in concentrations greatly exceeding that of ambient bottom water. We present here our measurements of concentrations of methane, hydrogen, carbon monoxide and nitrous oxide in the hydrothermal vent waters from the Galapagos Spreading Centre (GSC). The relationships of these constituents to silicon were unique for each vent field, with nitrous oxide at East of Eden being the only instance of negative correlation. These gases could serve as important energy sources, in addition to hydrogen sulphide, for the chemosynthetic bacteria which support the extensive and diverse animal population living in these environments[12,13].

Two principal types of hydrothermal vents have been discovered and studied to date. At the GSC (0°48′ N, 86°0.8′ W) warm (7–23 °C) water flowed from gaps, or 'vents', between basaltic pillows at linear flow rates of the order of cm s^{-1}; similar vents have been found at 21° N on the East Pacific Rise[14,15]. The second type, found at 21° N but not at the GSC, had hot (~350 °C) water flowing from sulphide 'chimneys' at rates of m s^{-1}. The data presented here represent hydrothermal water samples collected using the research submersible, *Alvin*, from three separate vent fields at the GSC in March 1979.

Corliss *et al.*[1] showed that the silica concentration in the GSC vent samples defined a single linear relationship with temperature and, owing to uncertainties in the temperature measurements, provided a better measure of the degree of mixing between the hydrothermal end-member and ambient seawater than did temperature. We therefore present the GSC gas data relative to dissolved silica in Fig. 1.

$CH_4/^3He$ and $H_2/^3He$ ratios for these vents are compared with those from sites along the East Pacific Rise in Table 1. The 21° N hydrothermal end-member is chemically very similar to the Galapagos end-member estimated by magnesium and silica geothermometry[3].

If the hydrothermal end-member solutions were in equilibrium with the quartz–fayalite–magnetite (QFM) buffer then they should contain approximately 400 mM hydrogen at 350 °C and 500 bar (ref. 18). This would imply $H_2/^3He$ ratios of the order of 10^{10} which are orders of magnitude higher than those observed (Table 1). Either the QFM buffer system does not control the H_2 concentration in these solutions or significant H_2 sinks, such as reduction of CO_2 and sulphate, occur in the ascending fluids. Hydrogen is an excellent energy source for several species of chemosynthetic bacteria[19], hence these reductions could take place abiotically or microbially in the warm water vents.

All of the reduced gases discussed here are produced both abiotically and microbially. Possible abiotic sources of methane, carbon monoxide and hydrogen include injection from the mantle and extraction from basalt by hot fluids. Additional abiotic sources include the reduction of carbon dioxide to form methane and the oxidation of magnetite to haematite,

$$2Fe_3O_4 + H_2O = 3Fe_2O_3 + H_2$$

which has been postulated as the major source of volcanic hydrogen[20]. Nitrous oxide could be produced abiotically by the reduction of nitrate in the descending seawater or by the oxidation of ammonium ascending with the hydrothermal fluids. Ammonium is known to substitute for alkali metal ions in silicate minerals and to be stable at high temperatures and pressures[21]. Nitrous oxide has also been detected in a few volcanic gas samples[22]. Carbon monoxide is a common constituent of volcanic gases[23] and could originate from C–O–H–S equilibria within the magma[24].

An alternative source of these gases in the hydrothermal fluids could be biological production. It is well established that methane, carbon monoxide, hydrogen and nitrous oxide are produced and consumed by microorganisms[25]. Hydrogen can be produced by many microbial species, methane by only one group, the methanogens. Little is known about the production of carbon monoxide by microorganisms but there is evidence that carbon monoxide may be an intermediate in methane oxidation[26].

Nitrous oxide is produced microbially through nitrification and denitrification. Both nitrifying and denitrifying bacteria

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have been isolated from water samples and animal guts taken from the GSC and 21° N vents (J. Baross, personal communication). Although nitrification and denitrification usually do not take place in sulphide bearing waters such as the vent waters, some bacteria, for example, *Thiobacillus denitrificans*[27], can reduce nitrate to nitrous oxide using sulphide as the electron donor. The nitrogen isotope data[28] indicate that the nitrogen found in vent animals is depleted in ^{15}N and its assimilation into animal tissue must have been preceded by bacterial nitrogen fixation and/or the bacterial assimilation of nitrate or ammonium.

We now consider the observation that nitrous oxide concentrations were found to be enhanced in some and depleted in other GSC vent waters compared with the ambient waters. In the ambient seawater, oxygen and nitrate are the principal electron acceptors involved in biological oxidation. Convective circulation within the warm water vent systems involves penetration of ambient water, thereby supplying these electron acceptors to the resident chemolithotrophic population[1,12,13]. In the process of nitrate reduction, nitrite and nitrous oxide can accumulate and/or serve as intermediate electron acceptors. East of Eden which had the highest observed sulphide to silica ratio (0.853) had the greatest residual potential for reducing electron acceptors. This is consistent with East of Eden nitrous oxide concentrations being lower than those of ambient seawater. Mussel Bed and Rose Garden had lower sulphide to silica ratios (0.072 and 0.232, respectively) and showed net production of nitrous oxide. The determining factor for net production or consumption of nitrous oxide is probably the local redox potential history of a water parcel within the more shallow convection cells of a given vent system.

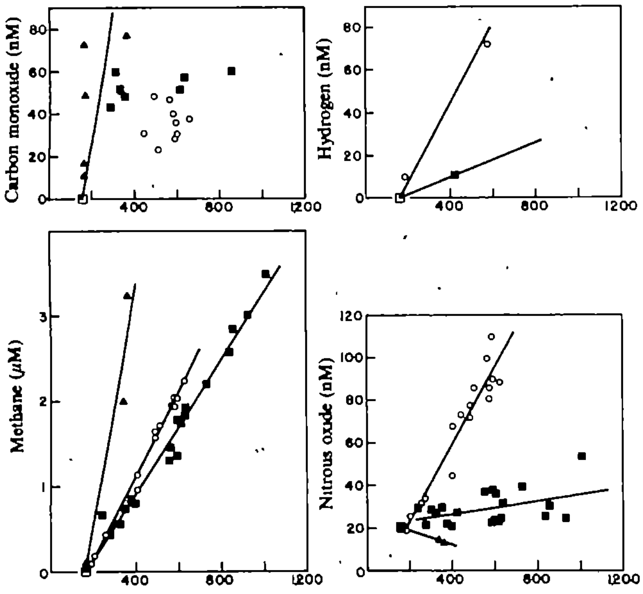

Fig. 1 Reduced gas correlations with silica for the GSC data. The locations and equations for the regression lines shown (not forced through the ambient seawater conditions) are:

△ East of Eden
$CH_4 = -2.23 + 0.0140\,Si$ $N_2O = 25.6 - 0.034\,Si$
○ Mussel Bed
$CH_4 = -0.82 + 0.0049\,Si$ $N_2O = -12.0 + 0.178\,Si$
■ Rose Garden
$CH_4 = -0.63 + 0.0039\,Si$ $N_2O = 19.8 + 0.016\,Si$

The regression line for CO is drawn for the East of Eden data only. Ambient bottom water concentrations were: CH_4, 0.3 nM; N_2O, 20.8 nM; CO, 0.3 nM; H_2, 0.4 nM. Each vent field was characterized by a different range of water temperatures and unique H_2S/Si mole ratios, namely △, 7–8 °C, 0.853; ○, 8–13 °C, 0.072; ■, 8–23 °C, 0.232 (J. Edmond, personal communication). All water samples, except those for H_2 which were taken in PVC samplers, were collected with the pumping system described by Corliss *et al.*[1]. These were subsampled into 125 ml tubular Pyrex flasks with Teflon stopcocks at either end and poisoned to 0.4 mM with $HgCl_2$. A modified version of the gas chromatographic procedure of Swinnerton and Linnenbom[16] was used. The gas chromatograph was equipped with helium ionization and flame ionization detectors.

Table 1 Ratios of hydrogen and methane to ^3He

Vent field	$CH_4/^3He$ (mole ratio)	$H_2/^3He$ (mole ratio)
Galapagos*		
East of Eden	$42.0 \pm 10.9 \times 10^6$	—
Mussel Bed	$14.8 \pm 1.8 \times 10^6$	$0.7 \pm 0.2 \times 10^6$
Rose Garden	$12.4 \pm 1.1 \times 10^6$	0.1×10^6
East Pacific Rise		
21° N, 109° W†	5.0×10^6	$22\text{–}43 \times 10^6$
20° S, 114° W‡	$5.8 - 17.4 \times 10^6$	—

* ^3He data for these GSC vents are not available. We assumed that ^3He in these vents does not differ appreciably from the values obtained by Jenkins *et al.*[7] for the GSC vents sampled during the 1977 expedition. ^3He values for our samples were derived by combining the ^3He-temperature relationship from their Fig. 2 (using the slope which included Garden of Eden) and the temperature-Si relationship for our samples: $T\,(°C) = \dfrac{Si(\mu m) - 28}{59.77}$ (J. Edmond, personal communication).

† Data from ref. 9.

‡ Data from ref. 17.

In addition to the variations seen in reduced gas concentrations among vent fields, systematic variations in several other chemical species have been reported in the GSC vent fields[1,3,5]. These may result from several causes, such as vent field age, differing substrate compositions and differences in water/rock ratios[4]. We hypothesize that the composition of the microbial assemblage in each vent field is characteristic of that field and this is reflected in characteristic production and consumption rates of the gases and other biogenous constituents in the vent water. This hypothesis is supported by the recent discovery that mixed-culture microbial populations, isolated from hot water and chimney rock samples taken at 21° N, can produce hydrogen, methane, carbon monoxide and nitrous oxide and consume methane at 100 °C (ref. 29).

Methane ^{13}C data indicate that the 21° N methane was abiogenic in origin[30] but ^{13}C measurements have not been done on GSC methane. However, because the $CH_4/^3He$ ratio at 21° N is considerably less than that in the cooler GSC vents, and as methanogens are present in vent samples at 21° N (ref. 29), we conclude that methanogens are likely present in the warm Galapagos vents and that part of the methane found in these vents is microbially produced. This is most clearly indicated at East of Eden which had the highest $CH_4/^3He$ ratio (Table 1) yet measured in these vent systems[17]. This conclusion is consistent with the reduced hydrogen levels in the GSC vents; hydrogen would be consumed in the microbial production of methane. Indeed, the question of possible microbial effects on the chemical constituents of submarine hydrothermal systems needs closer scrutiny.

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Palaeomagnetism of volcanic rocks of the Kodiak Islands indicates northward latitudinal displacement

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In the past decade it has become widely accepted that southern Alaska is composed of allochthonous terranes[1–4], but the times of amalgamation and their accretion to North America remain controversial. Palaeomagnetists and geologists generally agree that the Talkeetna superterrane, consisting of the Wrangellia and Peninsular terranes (Fig. 1), was far to the south of its present position with respect to North America in Triassic and Jurassic time[1–4]. This consensus is lost when it comes to the question of the relative position of these terranes in the Tertiary. Previously, limited palaeomagnetic evidence suggested that the Peninsular terrane may have lain substantially south of its present position[4], but the timing of deformational and thermal events and the apparent absence of a Tertiary suture zone argue for a Cretaceous docking of Wrangellia and the Peninsular terrane[5,6]. Here we present new palaeomagnetic results demonstrating that the Kodiak Islands were indeed at a more southerly latitude 62 Myr ago, around 43° N, and discuss the implications of this conclusion.

We sampled the volcanic rocks of the Palaeocene Ghost Rocks Formation exposed along the southeastern margin of the Kodiak Islands (Fig. 1). This turbidite sequence consists of a landward belt with intercalated flows of andesite pillows and a seaward belt including basalt flows. The sedimentary rocks are commonly isoclinally folded and locally grade into zones of tectonic disruption. The volcanic rocks are generally much less deformed and typically form coherent folds. The Ghost Rocks Formation is part of a late Cretaceous to Palaeogene accretionary sequence. The formation is apparently thrust to the north-west against a late Cretaceous trench turbidite sequence[7] and underthrust from the south-east by an Eocene to Oligocene submarine fan complex[8]. Metamorphic prehnite, pumpellyite and laumontite are locally developed[9]. The youngest foraminifera (Palaeocene) and 62 Myr radiometric ages of cross-cutting plutons suggest an early Palaeocene depositional age for the Ghost Rocks Formation.

We drilled and oriented cores of Ghost Rocks volcanics from two regions on Kodiak Island—Kiliuda Bay and Alitak Bay—which are separated by ~80 km along strike. In all, an average of seven cores were collected from each of 29 flows in the two regions. Flow attitudes were almost always obtained from interbedded sediments, and only occasionally from pillow morphology. The Kiliuda Bay sites included locations in both the andesite belt and the basalt belt, whereas the Alitak Bay sites included flows from the andesite belt only.

Stepwise thermal demagnetization proved effective in cleaning the samples from almost all of the flows. Characteristic components of natural remanent magnetization were isolated at temperatures of 300 °C and higher that trended towards the origin on vector diagrams. Considerable improvement in clustering of directions resulted for most flows: the average k increased from 15 to 40 during thermal cleaning. Only 2 of the 29 sites failed to clean up, and one of these was a highly deformed, badly fractured tuff.

Both polarities were found in the flows collected at Kiliuda Bay. Application of the F-test shows that normal and reverse mean directions are not significantly different from antiparallel. These directions also pass the fold test: when correction is made for the tectonic dip of the flows, k increases from 4 in geographical coordinates to 29 in stratigraphical coordinates (Table 1). At Alitak Bay only reversely magnetized flows are present, but these also pass the fold test (Table 1). Thus there is strong evidence that the characteristic magnetization of the rocks pre-dates folding in both areas and probably preserves the original field direction. Even after tectonic correction, however, the mean directions for the two regions are very different (Table 1). The mean declinations differ by 97°, suggesting the regions may have been rotated with respect to each other about a vertical axis. The inclinations also differ from each other by a much smaller, but still statistically significant amount. A likely explanation for this is that the Alitak Bay group of flows spans insufficient time to average out secular variation, although other explanations involving systematic differences in initial dips or in ages of flows between the two regions cannot be categorically ruled out.

The most important palaeomagnetic parameter for regional-scale tectonic interpretation is the mean inclination of the Palaeocene geomagnetic field for the Kodiak Islands. It is reasonable to assume that some sort of average of the data sets for the two regions would give a better estimate of the true inclination than either one of them alone. There are several techniques that have been developed for averaging inclinations only, when declination data are unavailable or should be excluded because of scatter (refs 10, 11, and A. Cox and R. Gordon, in preparation). A straightforward and simple way to do it for this case, however, is to rotate the sets of directions for both regions so that their mean declinations coincide (Fig. 2). Note that the resulting distribution of directions shown in Fig. 2 does not appear particularly non-fisherian (certainly it is not bimodal), which supports the idea that the discrepancy in inclinations between the two regions may be due to incomplete averaging of secular variation. Analysis of the combined

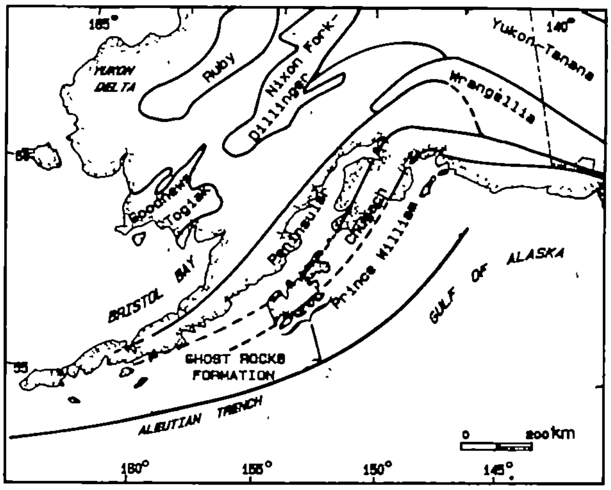

Fig. 1 Generalized terrane map of southern Alaska[14] showing location of the Ghost Rocks Formation.

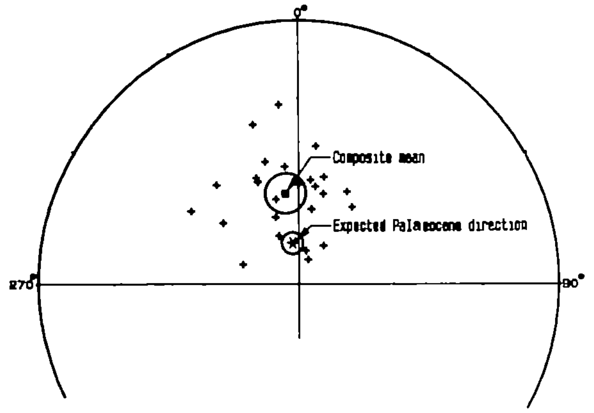

Fig. 2 Site mean directions (reversed inverted to normal): the mean declination for each region (Kiliuda Bay, Alitak Bay) has been rotated (31° clockwise, and 65° anticlockwide respectively) to coincide with the expected Palaeocene declination. Mean of composite data set (■) and expected Palaeocene direction (★) are shown with associated 95% confidence limits.

data set by Fisher statistics[12] gives a mean inclination of 61.4° and 95% confidence limits of ±6.5°. The same mean inclination, within a few tenths of a degree, is obtained by any of the more sophisticated inclination or colatitude statistics referred to above.

As shown in Fig. 2, the mean inclination of the Ghost Rocks volcanics is significantly shallower than that expected for Kodiak if it had been in its present position with respect to North America in Palaeocene time. The corresponding palaeolatitude is 43 ± 9° N, which is significantly less than the expected value of 66 ± 6° N derived from Palaeocene palaeomagnetic data for cratonic North America[13]. Thus the latitudinal displacement of Kodiak with respect to North America that is indicated is 23 ± 11° northward (Fig. 3); any longitudinal displacement is, of course, indeterminate.

The lithology, structural style and geological setting of the Ghost Rocks Formation suggest that it is a Palaeocene subduction complex. On the tectonostratigraphic map of Jones *et al.*[14] (Fig. 1) it is classified as part of the Prince William terrane. However, it is tied to the adjacent Chugach terrane (Fig. 1) by plutons of identical age (60–62 Myr) and composition. The only published palaeomagnetic data from the Chugach terrane are from two Eocene layered-gabbro plutons and a Cretaceous sheeted dyke and pillow basalt complex studied by Grommé and Hillhouse[15]. Although no fold test was described, their results from both sites suggest the Chugach terrane was about 25° south of its present position with respect to North America at these times, in excellent agreement with our results from the Prince William terrane.

The next terrane inboard is the Peninsular terrane (Fig. 1). It is simplest to suppose that the Peninsular terrane formed the backstop against which the late Cretaceous–early Tertiary accretionary complex of the Chugach–Prince William terranes formed, although the geological evidence for this is equivocal. Certainly very similar accretionary processes are occurring there today. The relatively few Tertiary palaeomagnetic data that have been published for the Peninsular terrane are consistent with this idea. In a study of 74 cores of lower Tertiary sediments from four closely spaced localities near Pavlov Bay, Stone and Packer[4] found shallow inclinations that imply low palaeolatitudes comparable to those we have found for the Ghost Rocks Formation.

Table 1 Palaeomagnetic results for Ghost Rocks volcanics

	Dec	Inc	A95	*k*	*N*
Kiliuda Bay					
Geographical	345.5	61.9	25.9	4.1	11*
Stratigraphical	320.1	52.7	8.5	29.5	11*
Alitak Bay					
Geographical	168.2	36.0	12.6	10.9	14†
Stratigraphical	56.7	68.3	8.6	22.6	14†
Ghost Rocks					
Mean	351.4	61.4	6.5	21.3	25

* Two sites eliminated because of unstable magnetization.

† Two superposed flows with identical NMR eliminated because directions were grossly discordant (>2 s.d. from mean).

Much further inboard, just north of the Denali fault, one finally encounters concordant palaeomagnetic directions. In a study of the Teklanika volcanics, Hillhouse and Grommé[16] have found steep inclinations consistent with the expected palaeocene field. Our own work on Ordovician limestones and a few early Tertiary lava flows in the Nixon Fork terrane 80 km to the west is consistent with their finding[17]. Thus, according to the palaeomagnetic evidence, considerable Tertiary convergence must have been taken up somewhere south of the Denali fault.

Figure 3 shows the band of possible Palaeocene positions for the southern terranes compatible with the palaeomagnetic results for the Ghost Rocks Formation. We have chosen our study to position the southern terranes in the early Tertiary because it is the only one on igneous rocks of well determined age and palaeohorizontal control that passes fold and reversal tests. The locations of the Pacific, Farallon and North American plate boundaries are essentially those of Engebretson (in preparation), who assumes fixed hotspots. The position of the Kula-Farallon ridge is less well constrained because the relevant magnetic anomalies have been subducted.

We favour a Palaeocene position of the southern terranes near to the Kula–Farallon ridge as drawn in Fig. 3 because other evidence suggests that the Ghost Rocks volcanics were the product of a ridge–trench encounter. These lavas are much too close to the subduction zone to be normal arc volcanism[18,19], and their composition, as well as that of the correlative pluton, suggests that they all have a mid-ocean ridge basalt parent[20,21]. Positioning the southern terranes north of the Kula-Farallon ridge allows them to be pushed northward by the movement of the Kula and Pacific plates, rather than being diverted to

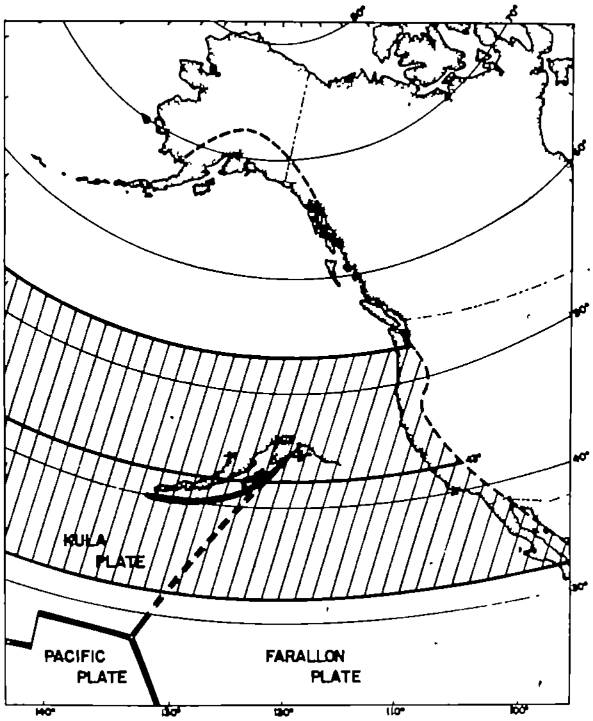

Fig. 3 Palaeocene reconstructiion showing palaeomagnetically inferred latitude for Ghost Rocks Formation, Kodiak, Alaska. Stippled palaeolatitudinal band is the uncertainty with respect to North America at the 95% confidence level. Heavy dashed lines suggest the Palaeocene North American plate margin.

the east by the motion of the Farallon plate. The mystery of the missing Tertiary suture zone in central Alaska required by this reconstruction, however, remains an enigma. These ideas and the data on which they are based will be presented in full detail elsewhere.

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^{40}Ar/^{39}Ar dating of pyrite

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The precise determination of the age of sulphide ore deposits is one of the most difficult problems in geochronology. This is largely because the very process responsible for concentrating certain metals to an economically significant level also tends to exclude radioactive isotopes from the deposits. It is customary to try to determine the age of mineralization by analysing silicate material presumed to be cogenetic[1]. Because such materials are not always available, and because it is not always clear that the silicates and ore minerals are in fact contemporaneous, we examined the sulphide minerals themselves to see if they contained sufficient potassium and argon for ^{40}Ar/^{39}Ar age determination. Our initial results indicate that this is the case for pyrite from the Geco ore body in northwestern Ontario, Canada.

The direct dating of sulphides with the Rb–Sr technique was attempted by Reesman and Hurley[2]. The results showed promise, but the approach was never pursued. More recently, Luck and Allègre[3] have shown that some deposits may be datable with the Re–Os method. Most promising is the pioneering work on the Rb–Sr dating of fluid inclusions in quartz from a tungsten deposit by Shepherd and Darbyshire[4].

Six pyrite samples have been analysed from the Geco mine, a stratabound and stratiform volcanogenic massive Cu–Zn sulphide deposit[5]. Geco has long been of interest to students of lead isotopes in connection with calculations of the age of the Earth and the chemical evolution of the mantle and crust[6]. The ore body has experienced the Kenoran orogeny[5] and therefore we had *a priori* information on its age. U–Pb and Rb–Sr dating in the area had shown[7] that the last severe regional metamorphism occurred 2,600–2,700 Myr ago. Furthermore, we could add a much more specific age constraint to the ore body when, during mineral separation, we discovered minute amounts of the easily datable biotite in intimate association with sphalerite crystals. A few flecks of these micas were therefore hand-picked for age determination along with the six pyrite samples.

Table 1 Analytical data for Geco biotites (step-heating)

T (°C)	Atmospheric (%)	^{40}Ar* ($\times10^{-5}$ cm^3 STP g^{-1})	*f*(39)	Age (Myr)
Sample GB250†				
810	1.2	6.21	3.7	2,530±31
910	0.2	42.80	24.3	2,589±3
1,100	0.2	55.51	30.8	2,620±3
1,600	0.2	73.54	41.3	2,604±2
Total	0.2	178.06	Integrated age	2,604±5
Standard $(40/39)_s$ = 43.57±0.19				
Mass = 0.0091g				
Sample GB1050				
550	52.8	0.08	0.2	1,137±142
650	24.8	0.17	0.5	802±104
700	5.9	0.88	0.5	2.426±41
780	2.3	2.78	1.4	2,614±8
820	0.4	21.94	11.5	2,600±5
950	0.1	73.51	37.4	2,637±3
1,170	0.1	89.78	45.8	2,633±4
1,600	2.0	5.21	2.7	2,630±11
Total	0.3	194.35	Integrated age	2,623±6
Standard $(40/39)_s$ = 45.22±0.32				
Mass = 0.0197g				

Decay constants from ref. 11 were used. Errors are ±1σ.
† Only negligible volumes of ^{40}Ar* were released from GB250 below 800 °C.

All samples were irradiated in the McMaster University reactor along with the Zartman hornblende standard[8]. Step-heating runs were performed on two biotite samples whilst the pyrites, because of their extremely low potassium concentrations (5–35 p.p.m.), were fused in single step analyses. The mass spectrometric isotope analysis of the evolved argon was carried out with an MS10 machine. The results are shown in Figs 1 and 2 and Tables 1 and 2. The biotite age spectra in Fig. 1 show excellent plateaus and there is evidence of only minute argon loss in the low temperature fractions. The plateaus are identical within experimental error and indicate that the cooling age of the ore body, after the Kenoran orogeny, is 2,610± 20 Myr. The age spectra indicate that the deposit has undergone negligible heating since then.

The pyrite data are shown in Fig. 2 where ^{40}Ar/^{36}Ar is plotted against ^{39}Ar/^{36}Ar. Such a graphical analysis must, of course, be used as there is no knowing *a priori* what the ^{40}Ar/^{36}Ar composition was in the pyrites at the time they cooled below their argon retention temperatures. The data points in Fig. 2 are clearly strongly linearly correlated, but the scatter about a straight line exceeds that due solely to expected experimental error. The scatter cannot be accounted for by system blank

Table 2 Analytical data for Geco pyrites

Sample	Mass (g)	K (p.p.m.)	^{40}Ar* ($\times10^{-7}$ cm^3 STP g^{-1})	^{40}Ar/^{36}Ar	^{39}Ar/^{36}Ar
OGS-10	0.2478	5.6	1.97	393.3±4.5	0.34±0.02
OGS-14	0.2613	8.8	2.56	444.4±2.8	0.54±0.01
OGS-131	0.8568	34.4	7.34	1,219.0±9.0	5.13±0.04
OGS-132	0.8484	16.8	3.72	840.9±15.5	2.88±0.02
OGS-133	0.8933	4.8	2.76	631.1±7.6	0.68±0.01
OGS-134	0.9348	11.9	3.06	521.4±6.3	0.56±0.01

Errors are ±1σ. ^{40}Ar/^{36}Ar and ^{39}Ar/^{36}Ar values have been corrected for a system blank of 1×10^{-8} cm^3 STP. The system blank has atmospheric argon composition.

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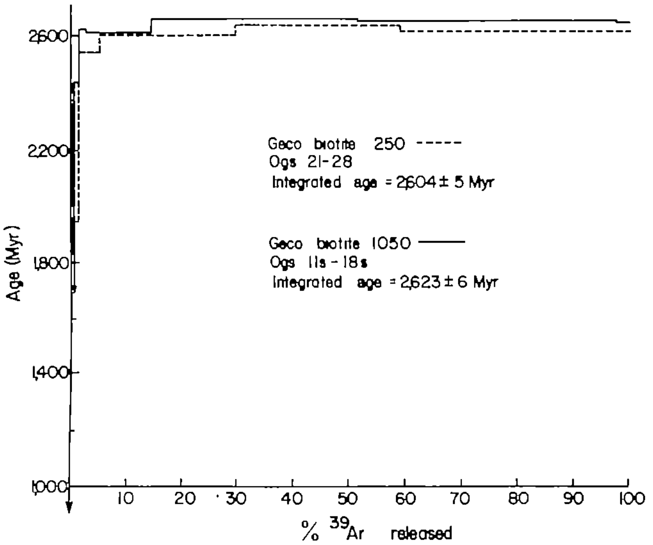

Fig. 1 Step heating data for Geco biotites. The age errors are listed in Table 1.

variation as the blank correction is no more than 5% of the plotted ratios. Thus enormous blank variations would have to be invoked for point OGS-134 (see Table 2).

When a least squares best line[9] is fitted to all the data, the slope corresponds to an age of 2,490±200(1σ) Myr, with an initial ^{40}Ar/^{36}Ar ratio of 369±28(1σ). When the most obviously discrepant point is omitted from the fit, the age and intercept estimates are scarcely changed, but the error estimates are significantly reduced. Thus the age becomes 2,500± 120 Myr, and the initial ^{40}Ar/^{36}Ar ratio is 359±17. The errors quoted in all cases correspond to the observed scatter of the points about the line.

The age indicated by the pyrites is thus indistinguishable, within errors, from that found for the micas, raising the distinct possibility of dating pyrite-bearing ore bodies directly with the ^{40}Ar/^{39}Ar technique despite the extremely low potassium levels. The reason for the scatter of the data in Fig. 2 is not yet clear. However, it is probably a reflection of a lack of homogeneity in the ^{40}Ar/^{36}Ar ratio among the pyrites at the onset of argon retention following the Kenoran orogeny.

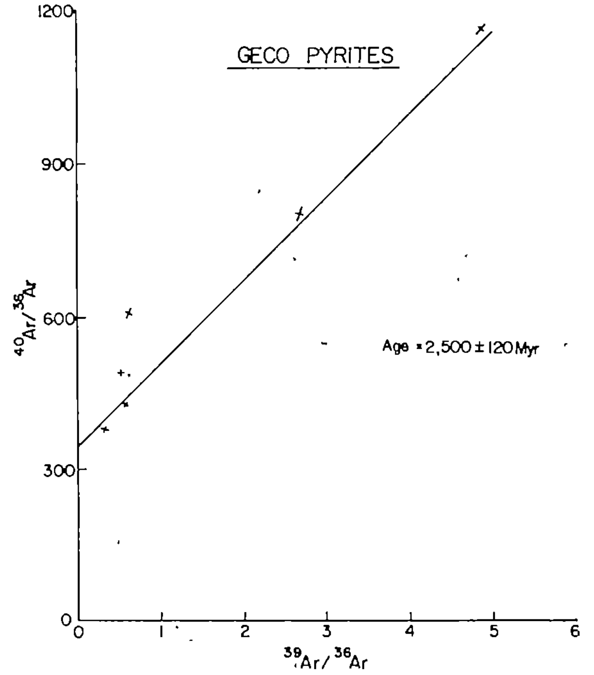

Fig. 2 Isotope correlation plot for Geco pyrites. The least squares best slope through all data points corresponds to an age of 2,490± 200 (1σ) Myr. When the most discrepant point is omited, the best slope corresponds to an age of 2,500±120 Myr. The error bars shown are the principal axes of the error ellipse about each point. The orientation is dependent on the correlation of the ^{40}Ar/^{36}Ar and ^{39}Ar/^{36}Ar errors. All error estimates are 1σ.

At this stage, it is not possible to say where the potassium and argon are located in the samples. All the pyrites were carefully hand-picked and examined to exclude external silicate contamination and the extremely low potassium concentrations found (Table 2) are testimony to the efficiency of this. However, there may have been minute potassium-bearing silicate inclusions completely encased within the pyrite. Some potassium and argon could be present in fluid inclusions, although the chlorine/potassium ratios (0.5–1) estimated from the ^{38}Ar/^{39}Ar ratios were not those characteristic of fluid inclusions ($\simeq$45) (ref. 10).

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Three-dimensional structure of a photosynthetic membrane

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The photosynthetic membranes in a variety of organisms are involved in the conversion of energy from sunlight into chemically useful forms[1–3]. *Rhodopseudomonas viridis* is a purple non-sulphur photosynthetic bacterium offering unique advantages for the study of photosynthetic membranes. Its internal photosynthetic membranes form large flat sheets which, as first described by Giesbrecht and Drews[4], contain subunits organized into a regular repeating pattern. The membranes are ideally suited to the use of Fourier image analysis techniques, and have been studied in two dimensions[5,6]. We have now determined the three-dimensional structure of photosynthetic membranes of *R. viridis* by electron microscopy. A large central structure protrudes from both surfaces of the membrane, and is surrounded by six smaller centres of mass. Comparison of these data with studies on the polypeptide composition of the membrane suggests a membrane in which a photosynthetic reaction centre is surrounded by light-harvesting bacteriochlorophyll protein complexes.

Membranes were isolated from pressure-disrupted cells as described elsewhere[7]. The membranes were stained with 2% aqueous uranyl acetate on carbon films and examined at tilt angles in an electron microscope ranging from 0° to 61° (Fig. 1). Densitometry and computer processing were carried out on selected micrographs to produce Fourier terms for computing the structure. Fourteen micrographs were used for the analysis, each of which provided as many as 22 individual measurements of amplitude and phase. Many of the micrographs showed regular detail in Fourier transforms extending to four orders (the lattice constant measured from these transforms and from electron diffraction patterns is 100 Å, corresponding to a centre-to-centre distance for membrane subunits of 115 Å),

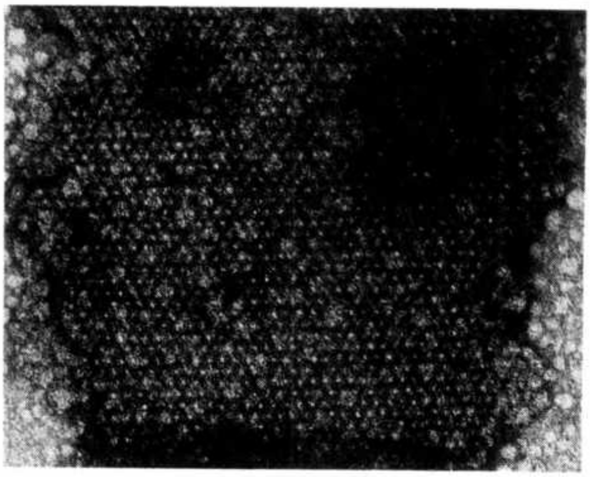

Fig. 1 Isolated photosynthetic membrane from *R. viridis*. The individual subunits can be seen arranged in a hexagonal lattice. The preparation was stained with 2% uranyl acetate. ×147,000.

indicating a resolution of 25 Å, but the final analysis was carried out to three orders for a resolution of 33 Å. Individual micrographs showed projected structure and Fourier terms consistent with the crystallographic space group P6, and the three-dimensional analysis was taken with P6 symmetry, at this level of resolution, applied to the data (see Fig. 2). The average phase residual determined from combining the individual images was 22 degrees, and all Fourier terms were considered in computing phase residuals.

To achieve a proper scaling of amplitude terms in the direction perpendicular to the plane of the membrane, 'edge-on' views of the membrane were also gathered. We used these images in a way similar to that described by Unwin and Zampighi[8] to calculate the 0, 0 lattice line through the origin of the three-dimensional Fourier transform.

The map calculated by these procedures highlights the stain-excluding structures of the membrane, and is subject to the usual difficulties and distortions associated with negative staining. An appropriate contour value was selected and used to produce an outer surface for the structure of each subunit in the membrane. The complete three-dimensional structure of such a subunit is shown in Fig. 3*a*.

A large central structure protrudes from each surface of the membrane, and a ring of smaller centres of mass surrounds it. The approximate width of the entire subunit is 100 Å, and the central region is approximately 70 Å thick. The central protrusion dominates the reconstructed image of the membrane; this domination can be appreciated from individual contour sections as well as from the surface map. Figure 3*b* shows a section through the subunit at the centre of the membrane, with the large central mass region clearly defined. The six 'satellite' regions are also visible, however, showing that the mass of the subunit is not completely restricted to one main region. The model agrees closely with images obtained from other techniques, including deep-etching and rotary shadowing (Fig. 4).

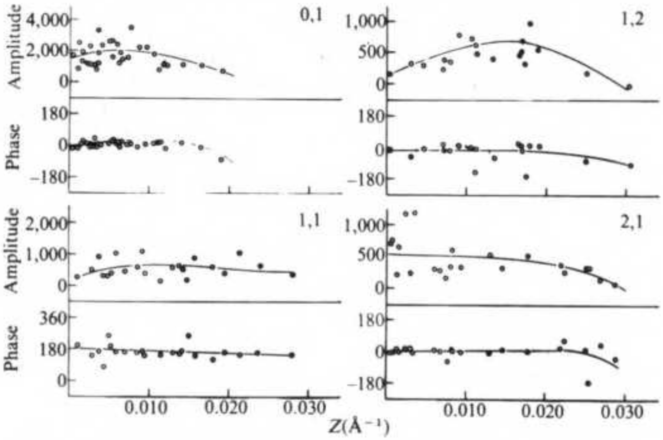

Fig. 2 Phase and amplitude plots of four major lattice lines used for the three-dimensional reconstruction. Values were sampled from these curves for the three-dimensional reconstruction.

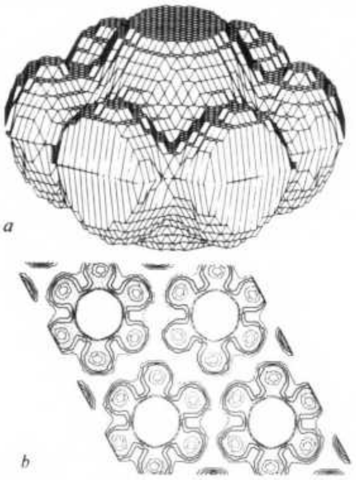

Fig. 3 *a*, Three-dimensional model of an individual membrane subunit. The surface was generated by applying a contour value to the reconstruction data and generating a surface envelope to fit the contour. A large central structure is seen which protrudes from each surface of the membrane, and six smaller lobes surround the protrusion. *b*, Four unit cells, arranged as they exist in the membrane, sectioned along a central plane parallel to the membrane sheet. Only the density of the central region can be seen (and not the projected density of the whole structure). In each subunit, the central region is surrounded by the six 'satellite' regions of mass density. Each central region is completely separated from its neighbour by a ring of such satellites.

Several workers have prepared reaction centres from *R. viridis* membranes[9,10]. X-ray and neutron diffraction studies[11,12] of similar reaction centres, from *Rhodopseudomonas sphaeroides*, in artificial lipid membranes, suggested an asymmetric mass distribution perpendicular to the membrane plane. The *R. viridis* membrane does not display such strong asymmetry, but note that we have used intact membranes rather than isolated reaction centres.

The *R. viridis* photosynthetic membrane contains light-harvesting components as well as the reaction centre apparatus[7,13,14]. A recent study in this laboratory revealed seven

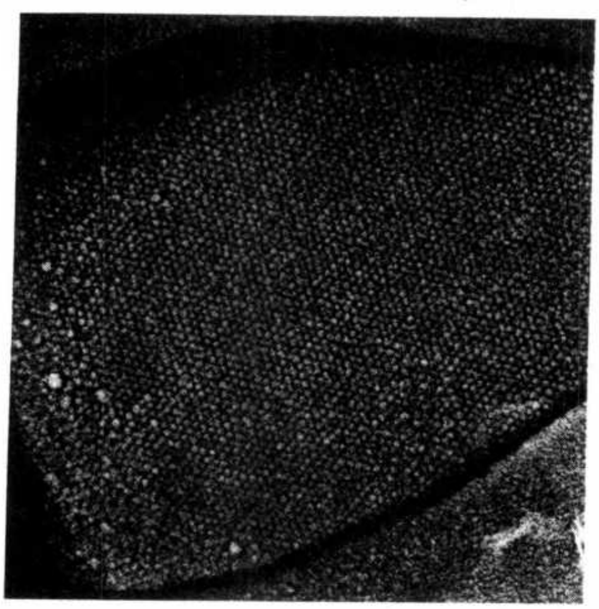

Fig. 4 Outer surface of the *R. viridis* photosynthetic membrane, exposed by deep-etching and rotary shadowing in a freeze-etching device. The lattice is clearly visible and individual subunits are well separated. ×100,000.

polypeptides associated with the intact membrane[7]. Two of the polypeptides (with apparent molecular weights of 11,000 and 16,000) bind bacteriochlorophyll and function in light-harvesting. Of the remaining five, three are associated with the reaction centre itself and two may have a role in electron transport[7]. Interestingly, an analysis of the apparent relative amounts of the seven polypeptides showed that the light-collecting components were present in 6–10 times higher concentration then the other five[7]. It thus seems reasonable to suggest that the 'satellites' of mass around the central structure are related to the two light-collecting components, and that the other five polypeptides (of molecular weights 24,000–44,000) are located in the central structure.

Can the membrane subunits visualized in this study accommodate the seven polypeptides associated with the membrane? We have estimated the volume of each membrane subunit at ~550,000 Å^3 (the volume of a disk 120 Å in diameter and 50 Å thick). Using a value of 1.3 Å^3 per dalton (derived from bacteriorhodopsin[15]), we might expect such a volume to accommodate approximately 423,000 daltons. Using the molar ratios for the seven membrane polypeptides reported by Jacob and Miller[7], we arrive at a value of 535,000 daltons per reaction complex. Although the molecular weight determination is slightly higher than the available volume, the negative staining used to prepare the samples for electron microscopy may have compressed the sample during the drying process, and could easily account for the slight difference. I therefore suggest that each subunit in the membrane contains a complete set (but only one) of reaction centre, electron transport and light-collecting components.

The programs used for data transformation and image recombination were kindly provided by Drs Linda Amos and Richard Henderson. A number of students and co-workers, including Jules Jacob and Yvonne Alvarez, helped with this project. Special assistance with data processing was provided by David Laidlaw, Tanya Falbel and David Salesin. I especially thank Drs Timothy Baker and David deRosier for the use of electron microscopy and densitometry equipment and for advice at several stages of this project. This work was supported by NIH grant GM-28799.

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Growth effects of SO_2 and/or NO_2 on woody plants and grasses during spring and summer

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The two most widespread air pollutants in Britain are SO_2 and NO_2 (refs 1, 2). These not only contribute to acidity in rain[3], which may seriously affect plant growth, but also cause substantial effects by their direct action as gaseous pollutants. Research into the effects of the gaseous pollutants on grasses has shown that the combination of SO_2 and NO_2 may be particularly toxic to plant growth[4,5]. Further research in this laboratory showed that *Phleum pratense* L. was more sensitive to SO_2 when growth was slow and it was suggested that exposures during winter may be particularly phytotoxic[6]. Here we present the results of experiments in which serious effects on plant growth were detected even during the summer months. Autumn-sown smooth stalked meadow grass (*Poa pratensis* L.) exposed to SO_2 or SO_2 plus NO_2 showed marked growth reductions during the winter and spring, and although the plants recovered during the summer, flowering was inhibited. Moreover, four broad-leaved tree species showed substantial decreases in growth when exposed to SO_2 plus NO_2 between March and August.

Experiments were done in four large hemispherical glasshouses situated outdoors, in which environmental conditions were close to ambient[7]. Pollutants were added to three of the glasshouses between 09.00 h on Monday and 17.00 h on Friday to give concentrations of 0.1 p.p.m. NO_2, 0.1 p.p.m. SO_2 and 0.1 p.p.m. $NO_2 + 0.1$ p.p.m. SO_2; weekly mean concentrations were 0.062 p.p.m. (Annual means of 0.06 p.p.m. SO_2 and 0.06 p.p.m. NO_2 may be experienced in several urban areas in Britain[8–10] where grasses and trees are grown for amenity purposes.) The fourth glasshouse (control) received partially filtered air without added pollutants.

The amenity grass *Poa pratensis* L. cv. Monopoly was exposed to SO_2 and/or NO_2 for 11 months, during which growth was monitored. Seeds were sown in each treatment on 2 October 1980 and after emergence 15 days later, 110 seedlings were potted into individual 7.5 cm pots containing John Innes no. 2 compost and placed at random within the relevant glasshouse. Samples of 10 plants per treatment were removed for harvesting at intervals until 7 September 1981. Plants were repotted into 10 cm pots in May, after which no further root weight measurements were made. The increases in control plant weight up to mid-May and in shoot weight at this and subsequent harvests are shown as natural logarithms in Fig. 1*a*; per cent reductions for each pollution treatment are illustrated in Fig. 1*b*. Between November and February, SO_2 and NO_2 appeared to interact causing greater than additive effects on plant weight; large reductions were evident in the combined gas treatment, while SO_2 alone caused only small reductions, and NO_2 alone promoted growth ($P < 0.05$). After this time, however, the interaction was lost and effects became approximately additive because small reductions developed in the presence of NO_2. During late spring and summer the polluted plants gradually recovered, until by September, shoot weight in NO_2 was similar to that in controls and that in SO_2 and $SO_2 + NO_2$ exceeded the controls by ~17% ($P < 0.05$). Culm development before flowering was first observed in mid-May and was followed by inflorescence

Table 1 Mean increases in total dry weight (g) of 14 seedlings and 14 cuttings of *Betula pendula* grown in clean air and in air containing 0.05 p.p.m. $SO_2 + 0.05$ p.p.m. NO_2

	Seedlings	Cuttings
Control		
Mean dry weight ± s.e.	4.30 ± 0.376	14.56 ± 0.553
c.v. (%)	32.7	14.2
$SO_2 + NO_2$		
Mean dry weight ± s.e.	3.11 ± 0.367	9.14 ± 0.373
c.v. (%)	44.2	15.3
Decrease in growth of polluted plants (%)	27.7	37.2
LSD between treatment and control means (%), $P = 0.05$	25.1	9.4

The percentage decrease in growth and the least significant differences (LSD) between the polluted and control means for the seedlings and cuttings are also shown.

development towards the end of the month; Fig. 1c shows mean numbers of culms per plant. SO_2, whether supplied alone or together with NO_2, not only delayed stem elongation but also reduced the total number of flower heads to less than half that on controls. The inhibition of flowering may have contributed to the recovery of shoot weight in these treatments by allowing vegetative growth to continue at faster rates than in the controls.

As little is known of the influence of SO_2 and NO_2 mixtures on tree growth, six species were selected for comparison. By using cuttings of only one genotype the variability between replicates can be reduced, and as only a few trees of each species could be accommodated in the fumigation chambers, this meant that small treatment effects would not be masked by plant variability. Therefore, second-year cuttings of *Malus domestica* Borkh. (apple MM 106), *Betula pendula* Roth. (silver birch), *Betula pubescens* Ehrh. (common birch), *Populus nigra* L. (black poplar), and *Alnus incana* L. (grey alder), and seedlings of *Tilia cordata* Mill. (small-leaved lime) were exposed to SO_2 and/or NO_2 from before bud break (23 March) and harvested after 150 days on 19 August 1981. Plants were grown in individual 19 cm Fyba pots containing J.I. no. 2, and were randomly positioned in each of the four glasshouses. The range of responses of the different species to SO_2 and/or NO_2 can be seen in Fig. 2, where the mean dry weights of the fumigated plants are expressed as percentages of the control means. In those species which showed reduced growth in response to pollutants, the combined treatment was the most toxic. There were interactive effects of SO_2 and NO_2 in *T. cordata*, *B. pendula*, *P. nigra* and *B. pubescens*, resulting in greater than additive responses. Those plants which were significantly affected in terms of dry weight also showed significant decreases in stem height and diameter. In some cases visible leaf blemishes were observed on the older leaves of polluted plants, and towards the end of the exposure period accelerated leaf senescence and abscission of the leaves occurred. The early loss of leaves from deciduous trees exposed to small concentrations of SO_2 is well known[11], and in the experiment reported here was found to be greater when NO_2 accompanied SO_2.

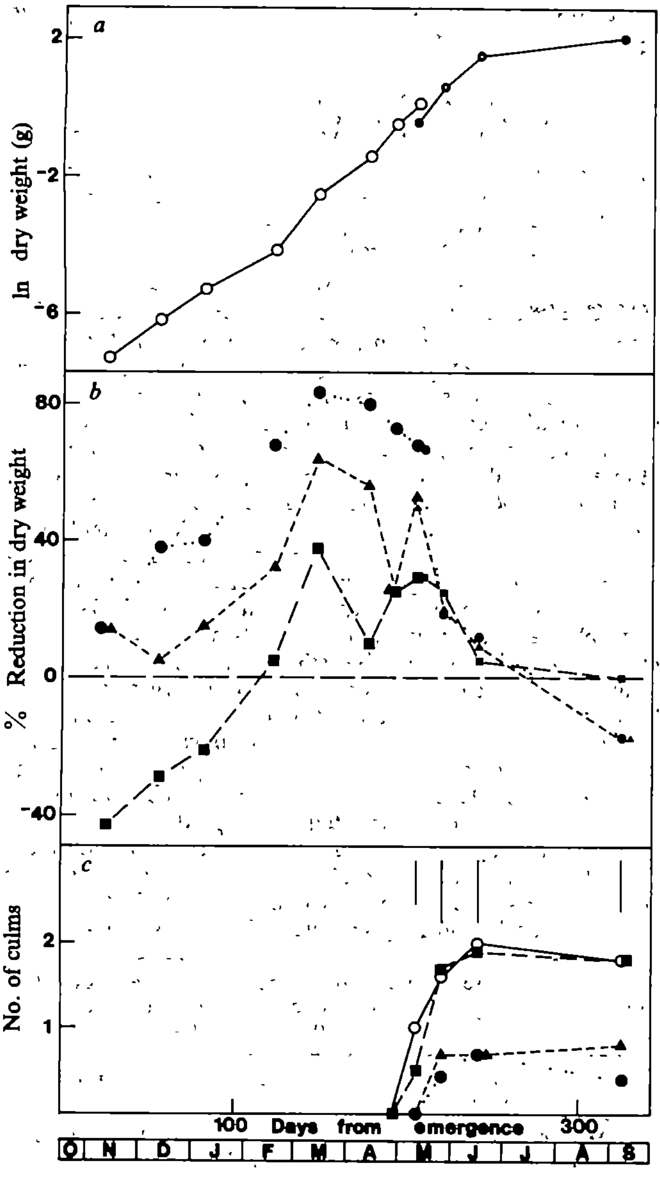

Fig. 1 *a*, Growth of *Poa pratensis* L. in partially filtered air. Plant weights are shown up to May (large symbols) and shoot weights at this and subsequent dates (small symbols). *b*, Percentage reductions in dry weight compared with controls after exposure of plants to weekly mean concentrations of 0.062 p.p.m. NO_2 (—■—), 0.062 p.p.m. SO_2 (– – ▲– –) or 0.062 p.p.m. SO_2 + 0.062 p.p.m. NO_2 (···●···). Large symbols represent effects on plant weight and small symbols effects on shoot weight. *c*, Development of culms: control (—○—), in NO_2 (—■—), and in SO_2 (– – ▲– –) or SO_2 + NO_2 (···●···). Bars represent LSD, $P = 0.05$.

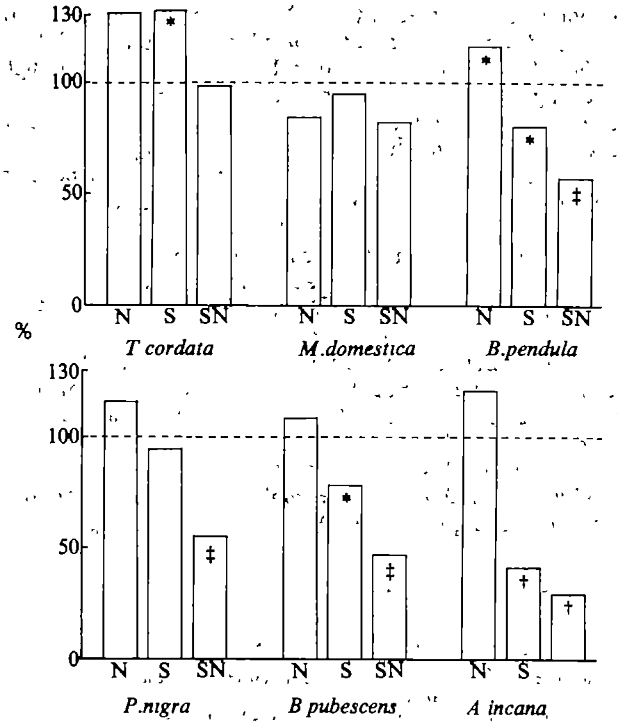

Fig. 2 The mean dry weights of woody plants ($n = 5$–8) exposed to weekly mean concentrations of 0.062 p.p.m. SO_2 (S), NO_2 (N), or $SO_2 + NO_2$ (SN) expressed as the percentage of the mean dry weights of controls grown in clean air. Treatment means that were significantly different from control means are marked: $^*P < 0.05$, $^†P < 0.01$, $^‡P < 0.001$.

To compare the effects of a $SO_2 + NO_2$ mixture on seedlings with effects on cuttings (of one genotype), a further experiment was conducted. Seeds of *B. pendula* collected in autumn 1979 were sown the following summer and cuttings from one seedling were bulked up by micropropagation; 14 seedlings and 14 cuttings were grown in two fumigation chambers from 27 May to 14 August 1981. The chambers were kept outside and were rapidly ventilated with charcoal-filtered air. Pollutants were added to one chamber to give an exposure concentration of 0.05 p.p.m. SO_2 + 0.05 p.p.m. NO_2. Table 1 shows the mean increases in dry weight of the seedlings and cuttings over the 80-day exposure period. The cuttings grew more quickly than the seedlings, but both groups of plants showed decreases in growth in air containing SO_2 and NO_2. The coefficient of variation (%) of each group is also shown and illustrates the greater variability in the growth of the seedlings. Clearly, a smaller treatment response could have been detected with the clonal plant material than with seedlings.

These data show that growth of deciduous species may be severely decreased by exposure to SO_2 and NO_2 mixtures during the growing season. Sensitivity clearly differed between species and the order of relative sensitivity coincided well with assessments made in the field by examining chronic injury at sites near to pollution sources[12]. Exposure to the individual gases caused stimulation of growth in *T. cordata*, *B. pendula* and in the grass *P. pratensis* at some harvests, which suggests that the pollutants provided additional sources of nutrients. Such responses have been reported previously for NO_2 and for SO_2 but are usually associated with conditions in which nutrient supply is limited[13,14].

The changing response to pollutants of the grass studied here illustrates some of the difficulties of predicting effects in the field and of interpreting results from an individual harvest. However, recovery of polluted plants during late spring and summer may not be observed in the field if plants are in competition with less sensitive species. An inhibition of flowering when plants are grown in SO_2-containing air may be of possible benefit in turf cultivation, but could have serious implications for commercial seed production.

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Falcon visual sensitivity to grating contrast

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The eye of a small falcon, the kestrel, is about half the size of the human eye and, if similar in all other respects, its visual acuity would also be expected to be about half that of the human. Here we report that the cut-off frequency (a measure of visual acuity) is twice the expected value and is the same for kestrel and human eyes. This remarkable visual performance is explained by anatomical features of the bird eye and the spherical pit of the deep fovea acting as the diverging element of a telephoto lens to magnify the image[1,2]. In the low-spatial frequency range, however, falcon contrast sensitivity is much less than that of humans for stationary patterns.

Experiments were carried out with a 2-year-old male American kestrel, *Falco sparvarius*, called Argus, hatched in the wild and acquired at about 1 month old. Stimuli were sinusoidal modulations of a homogeneous luminance field, that is, vertical spatial frequency gratings; electronically generated on the screen of a cathode ray tube (Tektronix 606; P31 phosphor). Mean luminance of the field was $\sim$40 cd m^{-2} (calibrated by Tektronix photometer, J16), and was unchanged by modulation. Grating contrast (depth of modulation) was defined as $(L_p - L_t)/2\bar{L}$ where L_p and L_t represent the luminance levels at the peak (maximum) and trough (minimum) of a spatial cycle, and $\bar{L}$ is the average luminance across the screen. Contrast was expressed on a logarithmic scale where contrast in decibels, dB, is equal to $20 \log_{10}$ (contrast). Two conditions were used: (1) stationary gratings with abrupt onset and offset and (2) phase changing gratings that abruptly reversed the sign of modulation every 2 s (0.25 Hz phase reversal). The screen subtended approximately 3 degrees of visual angle on the falcon eye at a distance of 200 cm. A thin vertical line (5 mm wide) bisected the screen into right and left fields. During a trial one side of the bipartite field contained the grating and the other side remained homogeneous at the same average luminance. Stimulus generation and control of the experimental procedure were completely computer-controlled (Digital Equipment Corporation; PDP-11/03).

The experimental apparatus consisted of three perches and a food dispenser. The computer was signalled when the kestrel landed on any one of the perches. The perch from which the stimulus was viewed, P_o, signalled that the kestrel was in the observing position, and initiated presentation of the stimulus. Two response perches, P_r and P_l, corresponding to either the right or left bipartite fields, respectively, were located 5 cm in front of and slightly below the stimulus screen, and signalled a response when pressed. The food tray was located directly above the screen and dispensed a small piece of beef heart when signalled by the computer.

The task for the kestrel was to initiate a trial by landing on P_o, decide if the grating was on the right or left side of the field, and fly to either P_r or P_l to indicate his choice. Argus appeared to scrutinize the stimulus field monocularly by turning his head first with one side towards the stimulus field and then the other before making his response flight. This suggests that the nasal (deep) fovea was used for the judgements required in this experiment. (The nasal fovea contains the highest density of photoreceptors and is used for distant viewing[1].) As soon as Argus left P_o the modulation was automatically removed from the screen to ensure that the response decision had been made at P_o. A food reward immediately followed each correct response. An incorrect response was followed by an auditory signal and the mean luminance was reduced to 0 for 50 s during which a new trial could not occur. Following the 'dark time' the screen returned to the mean luminance level as a ready signal for the next trial. Argus was allowed 100 s of viewing time on P_o before a trial was aborted and a new stimulus presented. A trial was also stopped if Argus left P_o and failed to arrive at either P_r or P_l within 3 s.

A two-alternative forced choice method of constant stimuli was used. The stimuli for each session consisted of one spatial frequency presented at four contrast levels each separated by 5 dB. The side of the screen receiving the modulation was randomly chosen, and the order of presentation of each of the stimuli was also randomized. The spatial and temporal stimulus conditions were selected in irregular order across sessions. Each session consisted of 200 trials, and all trials were included in the analysis. As the experimental procedure was automated, much of each session proceeded in the absence of an experimenter. Usually a session lasted 4–5 h. All sessions started in the early evening.

The probability of a correct response was plotted against stimulus contrast and the psychometric function was fit by eye. The contrast level associated with 75% performance was interpolated from the psychometric function to determine contrast

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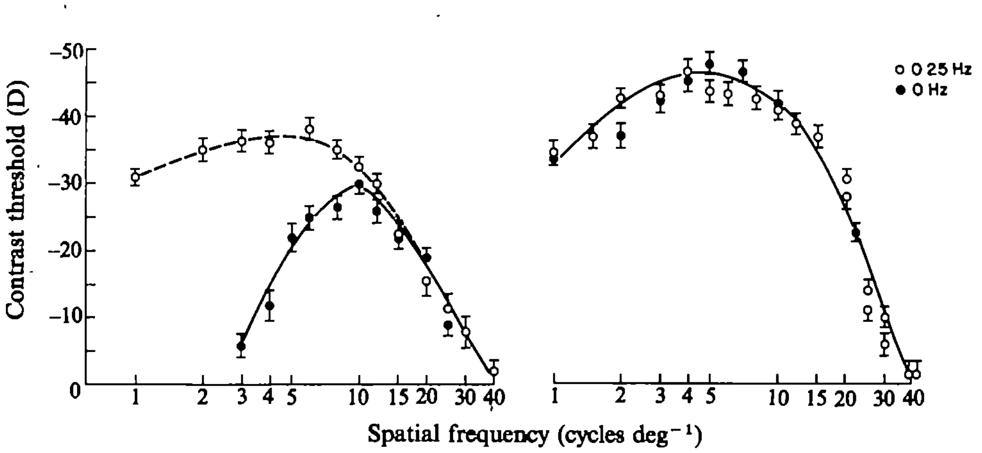

Fig. 1 Contrast threshold (dB) plotted against spatial frequency (cycles deg^{-1}) for falcon (*a*) and human (*b*) observers. Closed symbols represent thresholds for stationary gratings (0 Hz), and open symbols represent thresholds for gratings that abruptly reversed phase every 2 s (0.25 Hz). Data from three human observers (L.K., B.M. and J.H.) are combined on the human function. Error bars represent 1 s.d. of threshold estimates repeated for determination of reliability on about one-third of the data points.

threshold. A threshold was obtained only when the per cent of correct responses extended from 50% to ~90% or more. Many contrast thresholds were replicated at 4- and 6-month intervals to confirm stability of the measurements. A catch trial condition was periodically used where no pattern was presented on either side of the screen. Performance did not significantly differ from 50% in such conditions.

Contrast thresholds were similarly determined for three human observers (L.K., B.M. and J.H.) in the same experimental set-up as was used for Argus. Response keys were substituted for perches, and a synthesized voice unit (Votrax, VS-K) was substituted for the beef heart dispenser. The voice unit provided verbal feedback following each trial; otherwise, the software and data analysis for both experiments were identical. Viewing was binocular for the human observers.

Contrast at threshold was plotted against spatial frequency for both stimulus conditions and is shown in Fig. 1 for falcon and human observers. The data are presented so that the ascending direction of the ordinate represents decreasing contrast and, therefore, increasing sensitivity. The sensitivity of the falcon to stationary gratings was dramatically depressed below 10 cycles deg^{-1} relative to the phase changing gratings, suggesting that falcon vision may be highly dependent on temporal cues for discrimination of spatial frequency gratings below 10 cycles deg^{-1}. A similar low frequency fall-off has recently been reported for the contrast sensitivity function of a large eagle, *Aquila audax*[3]. Human contrast sensitivity functions were similar for both conditions. Absolute levels of peak contrast sensitivity (phase changing gratings) were approximately 0.5 log units lower for the kestrel than for the human. Increased photoreceptor noise, and/or reduced photon capture (possibly due, in part, to the small photoreceptors of the falcon eye) may account for the lower peak sensitivity.

Cut-off frequency for both human and falcon functions was ~40 cycles deg^{-1}. The human spatial cut-off frequency of 40 cycles deg^{-1} observed in this experiment is expected for the luminance level used here[4]. The results for the kestrel are inconsistent with a previous report that kestrel acuity is ~2.6 times higher than that of humans[5]. However, the similar cut-off frequencies for both human and falcon reported here can be explained in terms of the known geometry of the falcon eye and retina.

The falconiform eye is distinguished from other vertebrate eyes by a two-foveal retina[1,6,7], closely-packed foveal photoreceptors[1,2] and superior optics[8]. In general, cut-off frequency of an eye can be estimated from focal length, photoreceptor spacing and pupil diameter[9,10]. Pupil diameter, measured periodically during the experiment by placing a rule about 1 cm away from the eye while Argus was on the observer perch, was ~2.5–3.0 mm, and thus sufficient to pass the spatial frequencies used in this experiment[2]. In falconiform eyes, focal length, F, is ~0.65 of the axial length, L, and can be estimated from head width, W, where $W = 2L$[1,11]. Head measurements made with calipers indicate that $W = 28$ mm for Argus. Thus $F = 9.1$ mm, which is about half the human focal length[10], and therefore limits acuity by a factor of half relative to that of humans. However, centre-to-centre spacing of falconiform foveal photoreceptors, d_{cc}, is 2 μm (refs 1, 2), which is two-thirds of the human value and provides a 1.5 factor acuity advantage for the falconiform. Based on focal length and centre-to-centre spacing of photoreceptors, the predicted cut-off frequency for the falcon would be three-quarters of the observed human cut-off frequency or 30 cycles deg^{-1} against an observed cut-off frequency of 40 cycles deg^{-1}. The boost in falconiform spatial frequency cut-off can be accounted for by an image magnification factor m, as given in the equation[2]

$$\nu = \frac{F}{d_{cc}\sqrt{3} \times 57.3} \times \mathrm{m}$$

where ν is the spatial cut-off frequency in cycles deg^{-1}, and F and d_{cc} are as defined above. If m, the foveal magnification factor, is set equal to 1 for humans then the equation gives a magnification factor of 1.33 for the falcon. Magnification factors due to the spherical pit of the deep fovea have been estimated to be ~1.10–1.25 for the eye of a jumping spider (Salticidae)[12] and 1.45 for a human-size falconiform eye[1]. The magnification factor of 1.33 reported here is consistent with previous estimates and thus may explain the extraordinarily high visual acuity of the falcon given both the advantage of small photoreceptors and the limitation of small eye size.

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Gigaseal patch clamp recordings from outer segments of intact retinal rods

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The hyperpolarizing light response of vertebrate photoreceptors depends on cation-selective ionic channels in the cell membrane, which open in darkness and close in light. Little is known about these channels even though they are crucial elements in the transduction of light into a change in receptor potential. Here we report our attempts to study the light-sensitive channel using a patch pipette technique for recording ionic currents from a small area of outer segment membrane. The electrode was sealed against the membrane with a sealing resistance higher than a gigaohm, permitting good resolution of membrane currents[1,2]. Working at low external calcium concentrations this allowed us to observe in response to light small outward currents (the difference currents observed when the light-sensitive channel closes, thus switching-off an inward dark current), which reversed polarity with membrane depolarization. We were unable to recognize discrete current steps that might represent single channel openings and closings modulated by light. However, the light-induced outward current was accompanied by a decrease in membrane current noise. Analysis showed that the elementary event in 0.1 mM external calcium is ~12 fA. If it is assumed that the elementary event is due to the closure of a single light-sensitive channel and that the potentiating effect of low external calcium on the photocurrent is due solely to an increase in single-channel current, one can calculate that in normal calcium the value of the elementary event is 2 fA, corresponding to a single channel conductance of 50 fS. A conductance of this size is consistent with ion permeation via either a mobile carrier or a pore.

Experiments were performed on the dark-adapted retina of the nocturnal lizard, *Gekko gekko*, which was chosen after unsuccessful attempts to obtain stable patch recordings from other types of photoreceptors. The recording method is shown in Fig. 1*a*. A piece of retina from approximately one-fifth of the eye was isolated and placed in a perfusion chamber with the photoreceptor outer segments oriented parallel to the stage of an inverted microscope. Under visual observation through an IR image converter, the recording pipette was introduced into the chamber from the side and pressed against either the tip or, less frequently, the distal side of the outer segment of single intact rods. For ~60% of the time, brief suction applied to the pipette caused its resistance to increase suddenly from ~4 MΩ to 1–50 GΩ, as has been shown with other tissues[1,2]. Seal resistances in the gigaohm range provide an order of magnitude improvement in current resolution over the recordings obtained using the rod 'sucking' technique[3,4], and allow us to apply voltage steps to the patch without penetrating the cell with microelectrodes.

Over the course of these experiments, gigaseal recordings were obtained from 202 rods, and 49 of these responded to light with an outward change in membrane current. The reason for the low percentage of responding cells is not known. The membrane at the tip of the outer segment, which is shed during receptor renewal, might be abnormally fragile or not always functional. It is also possible that the low yield of responding cells is due either to damage sustained in preparing the isolated retina or to breakage of the outer segment membrane when suction was applied in attempting to form a gigaseal. The incidence of membrane breakage was highest in low Ca^{2+} solutions, which set a practical limit to the amount by which the external calcium concentration could be reduced.

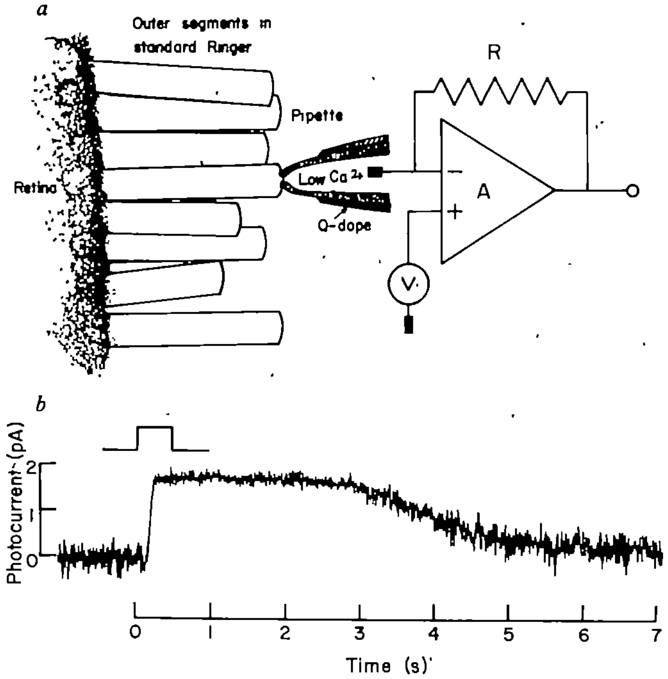

Fig. 1 *a*, Recording method. The isolated retina was anchored in a chamber through which flowed a continuous stream of oxygenated and chilled (~18 °C) Ringer's solution. A fire-polished glass pipette insulated with polystyrene Q-dope (GC Electronics) and filled with low calcium Ringer's solution, was pressed against the tip of a suitable rod outer segment Gentle suction was briefly applied to the inside of the pipette until the seal resistance abruptly increased to >1 GΩ. Ag/AgCl pellet electrodes were used in the pipette and bath. Current flowing across the patch of membrane at the pipette tip was measured with a virtual ground circuit using a 10 GΩ feedback resistor (R). The potential inside the pipette was controlled by a variable voltage source (V). The output of the amplifier (A) was low-pass filtered at 100 Hz or 160 Hz (8 pole), amplified further and recorded on an FM tape recorder. For all experiments, the Ringer's solution contained (mM): NaCl, 159; KCl, 3.3; $CaCl_2$, 1.0; $MgSO_4$, 1.0; dextrose, 10; HEPES, 2.8 (neutralized with NaOH to *p*H 7.8). The pipette filling solution was the same except $CaCl_2$ was 0.1 mM. *b*, Light response of a patch of rod outer segment. The trace shows the recording of membrane current (outward current positive) before and after a 500-ms flash of white light (photocell trace on top); bandwidth d.c. to 100 Hz. Stimulus intensity was 6.25×10^{-10} erg μm^{-2} $flash^{-1}$. Seal resistance 1 GΩ.

Figure 1*b* shows the response of a gekko rod to a moderately bright flash of white light. The outward photocurrent rose rapidly to a peak amplitude which persisted for a few seconds and then declined slowly. The size of the photocurrent produced by saturating light intensities varied from cell to cell over about a tenfold range, with a mean value of 1.6 pA. For the cells having the largest photocurrents, it was possible to see that a protrusion of outer segment membrane had been drawn into the tip of the patch pipette. Since recordings of this type differed from the others only in the size of their light response, we attributed the variations in photocurrent amplitudes to differences in the area of membrane in the patch pipette.

To establish that the observed photocurrents were due to changes in the conductance of the membrane under the recording pipette, we examined the influence of the patch potential on the light response. The potential across the membrane patch was changed by applying the appropriate voltage to the external surface of the membrane patch via the pipette. The inside of the pipette was made negative for depolarization and positive for hyperpolarization. The outward photocurrent observed at resting potential increased in amplitude with hyperpolarization,

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decreased with depolarization, and appeared as an inward current at potentials displaced by more than approximately +40 mV from the resting potential. Detailed analysis of these experiments is complicated by the fact that the intracellular potential changes during the light response, as the whole outer segment was illuminated. Nonetheless, the results provide evidence that the current response is due to a light-induced conductance change of the membrane patch with a reversal potential of about 0 mV.

The current recorded from a patch of outer segment membrane fluctuated in darkness in an apparently random manner. The peak-to-peak noise in the quietest recordings was 0.1–0.2 pA and contained no recognizable discrete current events that could be considered the opening or closing of single light-sensitive channels. In all cells, steady illumination reduced the current fluctuations. For example, in the experiment of Fig. 2, the current fluctuated over a range of 0.5 pA (peak-in-peak) in darkness with a mean variance of 1.93×10^{-26} A². Steady illumination with a moderate light then produced a maintained outward photocurrent of 0.67 pA, and the mean current variance was reduced to 0.98×10^{-26} A². When the light was turned off, the outward photocurrent declined and the noise recovered slowly over the following 60 s to a final mean value of 1.69×10^{-26} A². The change in variance caused by steady light was calculated by subtracting the grand average of the variances during light from the grand average of the variances measured during the two dark periods. Based on eight cells for which it was possible to obtain sufficiently long and stable recordings during the dark–light–dark sequence, the average value of the reduction of noise by steady light was 1.9×10^{-26} pA². The approximate magnitude of the elementary events underlying the light-suppressed noise was estimated by dividing the change in current variance by the change in mean current[5]. This calculated unit event size did not depend on the intensity of steady illumination, nor did it correlate with the magnitude of the saturating light response produced by the cell. In the eight cells referred to above, the amplitude of the estimated unit event ranged from 4 to 21 fA, with a mean value of 12 fA.

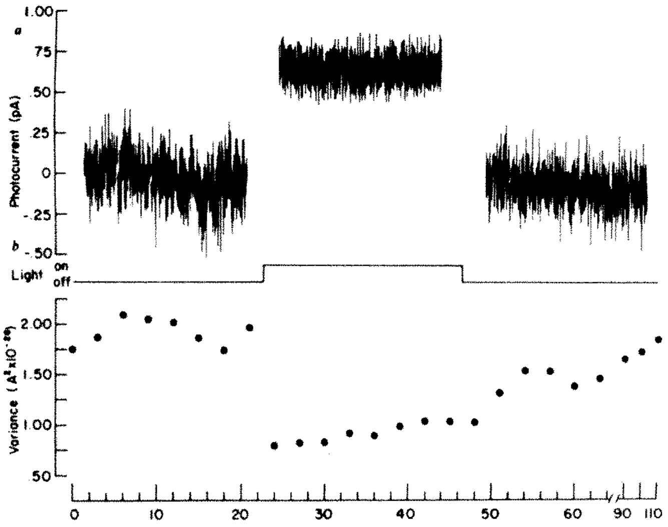

Fig. 2. *a*, Recordings from an outer segment patch in darkness and during steady illumination. The traces shown are representative 2-s long samples of the high gain current record before, during and after steady illumination. The steady outward photocurrent produced by illumination is indicated by the vertical displacement of the trace obtained in light. The output of the light monitor is shown on the middle trace and applies to both parts *a* and *b*. *b*, Graph of the current variance versus time. The continuous current trace was divided into successive 2-s long segments. A laboratory computer (DEC LSI 11/23) was used to digitize signals to form 1,024 points at 2-ms intervals, and to calculate the variance within each 2-s long segment[14]. The dark values in the text are the averages of a larger number of values than are shown in the graph. The return of noise following illumination was initially sluggish, approximating the initial dark level 45–60 s after the return to darkness (note break in the time axis). Bandwidth d.c. to 160 Hz; stimulus intensity 326 photons μm^{-2} s^{-1} at 520 nm; seal resistance 3 GΩ.

Our results do not allow us to decide what the unit event represents. It could be the change in membrane current due to the closure of a single light-sensitive channel, or the spontaneous release of a packet of transmitter substance resulting in the fairly synchronous closure of a number of channels. If we assume that the unit event we have measured is due to the closure of a single light-sensitive channel, we can make several inferences about the properties of the channel. The recordings described here were obtained in an external medium containing 1/10th of the normal calcium concentration. Previous measurements in rods have shown that lowering the external calcium concentration increases the light-sensitive current[6–9]. Decreasing the external calcium concentration from 1 to 0.1 mM is associated with an approximate fivefold increase in photocurrent[9]. The mechanism of this effect is unknown; however, it could be that low calcium concentrations increase the single channel conductance and/or the number of functioning light-sensitive channels. Preliminary experiments have not allowed us to choose between these alternatives. It is interesting, however, that if external calcium influenced the photocurrent solely through an action on the conductance of light-sensitive channels, then the estimated unitary event in normal calcium would drop to 2 fA. This value agrees remarkably well with the shot event size calculated on more theoretical grounds for toad rods[10]. With a unit event of this size, a 1 pA single-photon response would require the simultaneous closure of ~500 channels. Furthermore, if the maximum photocurrent produced by the outer segment is between 20 and 30 pA (ref. 4) than the total number of light-sensitive channels would range over 10,000–15,000 per outer segment, giving a channel density of 10–15 μm^{-2}.

The experiments that measured the reversal potential of the light response indicate that the driving force on the light-sensitive channel is ~40 mV. A unit event of 2 fA would then correspond to a single channel conductance of 50 fS. This is ~140 times smaller than the conductance of a single electrically excitable sodium channel[11]. The flux through a channel this size would be 1.25×10^4 sodium ions per second, which is of the same order as that for a shuttle-type mobile carrier like valinomycin[12,13]. It has been reported[9] that in 0 Ca-EGTA the photocurrent increases approximately 20-fold. If we retain the assumption that external calcium influences solely the single-channel current then the flux through the channel could be as large as 2.5×10^5 ions per s. This rate of ion permeation is intermediate between what could be accomplished by a carrier and what is clearly a pore. The argument for a mobile carrier would be strengthened if the unit event we have measured involved a number of permeation sites. Our results are thus compatible with either a carrier or a pore being responsible for ion movement through light-sensitive channels in the rod outer segment.

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Cholecystokinin activation of single-channel currents is mediated by internal messenger in pancreatic acinar cells

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Cholecystokinin (CCK) is one of several active peptides that have been isolated from both the gut[1] and the brain[2]. CCK evokes membrane depolarization and conductance increase in both pancreatic acinar cells[3,4] and pyramidal neurones of hippocampus[5]. In the acinar cells, activation of cholinergic muscarinic and two different peptidergic receptor sites (for CCK and bombesin) result in the same type of conductance increase[6]. The application of intracellular Ca mimics the action of the agonists[7] and Ca has been proposed as an intracellular mediator for the membrane conductance increase[8]. A cation channel activated by internal Ca has been directly demonstrated in single-channel current recording experiments on isolated patches of plasma membrane from the basolateral surface of pancreatic acini[9]. We now demonstrate that CCK and acetylcholine (ACh) can activate these inward current channels indirectly. Single-channel currents were observed in electrically isolated membrane patches *in situ* when micropipette application of CCK or ACh was made outside the patch area. Single-channel currents from the same membrane patches were subsequently recorded after they had been excised (inside-out) and identical values for the single-channel conductance were obtained (~35 pS). No effect of CCK was obtained in the presence of the specific receptor antagonist dibutyryl cyclic GMP[10–12] although in all such cases single-channel currents were subsequently recorded from the excised patch (Ca-activated). These results demonstrate directly that CCK and ACh control ion channel opening via an intracellular messenger (Ca).

We used collagenase-isolated mouse pancreatic acini prepared as described previously[13,14]. Single-channel current recordings from the basolateral plasma membrane were made *in situ* or in excised membrane patches (Fig. 1) using the techniques described by Neher and collaborators[15,16]. The acini were stimulated by local micropipette application of ACh or cholecystokinin-octapeptide (CCK$_8$; Cambridge Research Biochemicals, CCK$_{26-33}$ octapeptide amide, sulphated) in the presence or absence of the specific CCK antagonist, dibutyryl cyclic GMP (Sigma)[10–12].

The experiments always began by recording from patches *in situ* as shown in Fig. 1*a*. Good seals between pipette and plasma membrane (>20 GΩ) were obtained in patches from 25 batches of mouse pancreatic acini. In the absence of agonist the patches were, in most experiments, completely silent (Fig. 2*a*). Spontaneous unitary currents were only observed in two patches; these were not studied further. Patches were sometimes monitored for long periods (up to 20 min) without stimulation. The initially silent patches did not subsequently develop spontaneous activity. In experiments designed to investigate the effect of CCK, this agonist was delivered by diffusion of the hormone from the tip of a micropipette brought close to the acinar unit from which high resolution current recording was made (Fig. 1*a*). Because the CCK-containing pipette was moved gradually until the tip was at a distance of about 20 μm from the site of the electrically isolated patch (an inverted microscope with ×500 magnification was used), it was impossible to obtain very accurate values for the latency of activation of the single-channel currents evoked (Fig. 2*b–e*). The activity normally appeared 20–40 s after the pipette had reached its final position. In six experiments on six different membrane patches, CCK stimulation resulted in the appearance of unitary current steps of the type shown in Fig. 2*b–g*. The recordings were very stable and currents at many potential levels could be monitored in each individual experiment. The linear single-channel current–voltage (*I–V*) relationship (see Fig. 3*a*) gave a 35-pS single-channel conductance. After a suitable amount of data had been recorded at various potential levels, the patch pipette was withdrawn from the acinus and the tip passed through the bath solution–air interface once or twice to form an inside-out membrane patch[16] (Fig. 1*b*). High resolution current recording was continued and unitary current steps were always observed in this condition (Fig. 2*h*). Again, currents were recorded over a wide range of membrane potentials. The current–voltage relationships shown in Fig. 3*a* make clear that the single-channel conductance obtained in the excised patches is virtually the same as *in situ*. In the isolated patches, reversal of current polarity always occurred at zero pipette potential (symmetrical solutions) where *in situ*, reversal occurred at a pipette potential of −25 mV (Fig. 3*a*). A separate series of four experiments on excised patches in which normal ionic gradients were present (physiological saline with 'extracellular' composition in pipette and 'intracellular' fluid in bath) was also carried out. In these experiments, in which the transmembrane ionic gradients correspond to the *in situ* recording situation, the usual type of unitary current activity was seen (Fig. 2*i*, *j*) and *I–V* relationships were obtained (Fig. 3*b*). The single-channel conductance was the same as in the other experiments, with current

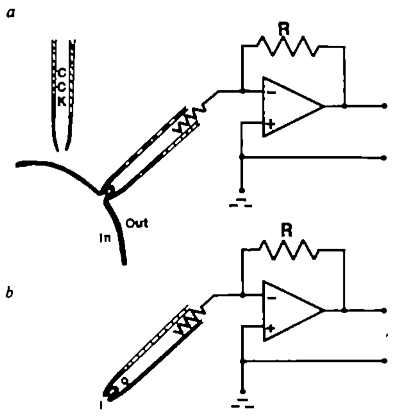

Fig. 1 Schematic outline of experimental system *a*, Recording from *in situ* membrane patch. Fabrication of patch pipettes and recording system as previously described by Neher and collaborators[15,16]. In these experiments the recording pipette was always filled with the bath solution, which had the following composition (mM): 140 NaCl, 4.7 KCl, 2.5 CaCl$_2$, 1 13 MgCl$_2$, 10 glucose and 10 HEPES. The solution was titrated to *p*H 7.3 by NaOH No agonist was present in the pipette solution. The pipette labelled CCK contained the octapeptide in a concentration of 5 μM dissolved in 0 9% NaCl acidified to a *p*H of 4 0 by HCl No currents were applied to this pipette It has previously been shown that glass micropipettes filled with the CCK analogue caerulein will cause substantial depolarization of mouse pancreatic acinar cells (microelectrode experiments) when the tip is placed close to the outside of an acinus[24] Similar experiences have been gained with CCK pipettes In this work the spontaneous diffusion of CCK from the tip of a micropipette was therefore used as the means of stimulation. In experiments with ACh stimulation the pipette was filled with 2 M ACh–Cl A braking current of 20 nA was used. During stimulation an ejecting current of 500–1,000 nA was applied. *b*, After the *in situ* recording the patch-clamp pipette was withdrawn from the acinus under investigation and the tip passed through the bath fluid–air interface to obtain an excised inside-out membranne patch[16] In these experiments single-channel recordings were made in symmetrical saline solutions. In other experiments the bath fluid was changed to one having an intracellular composition (mM) of 145 KCl, 10 NaCl, 1 13 MgCl$_2$, 10 glucose, 10 HEPES and 10 μM CaCl$_2$ with *p*H of 7.3 This enabled single-channel currents from excised patches to be recorded in conditions with normal transmembrane ionic gradients

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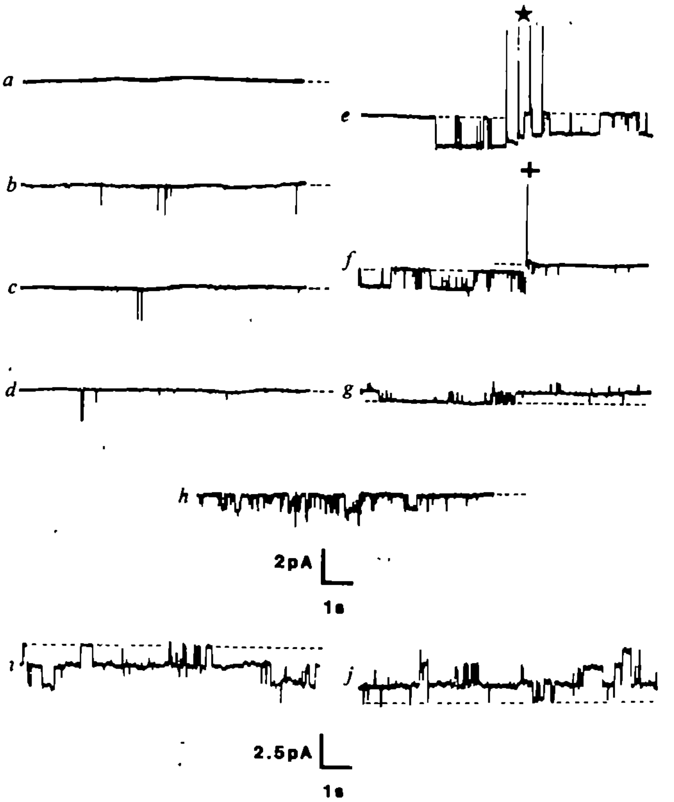

Fig. 2 The effect of CCK application. *a–h*, Consecutive traces from same membrane patch. *a*, Recording situation as described in Fig. 1*a* legend, but with the CCK pipette far away from the acinus under investigation. Pipette potential +40 mV. *b–d*, Three continuous traces obtained 20, 30 and 40 s, respectively, after the tip of the CCK pipette had been brought close to the patch pipette (recording situation as shown in Fig. 1*a*). Pipette potential +40 mV. *e*, 50 s after start of CCK stimulation. At the asterisk the potential of the patch pipette was changed (in steps of 5 mV) from +40 to +20 mV. *f*, Pipette potential +20 mV. At the plus sign the potential was changed to 0. *g*, Pipette potential −40 mV. *h*, Currents from the same patch after excision (inside-out) (recording situation as shown in Fig. 1*b*). Symmetrical extracellular saline solutions. Pipette potential +50 mV. *i*, *j* show currents recorded from another excised (inside-out) membrane patch with intracellular solution (high K, low Na) in the bath and the normal extracellular solution in the pipette. Pipette potential +60 mV in *i* and −60 mV in *j*. Dashed lines represent the current level when all channels are closed. Downward deflections represent the current from extracellular to intracellular side of membrane

reversal occurring at a pipette potential of −5 mV (Fig. 3*b*). The difference between this reversal potential and the reversal potential obtained in the *in situ* recording situation (−25 mV) indicates the existence of a 20 mV inside negativity in the intact acini during CCK stimulation. This corresponds reasonably well to values obtained with microelectrodes[3].

The effect of CCK application was also investigated in a series of experiments in which a specific CCK antagonist (dibutyryl cyclic GMP)[10–12] was present in the bath solution. In none of the six experiments carried out did CCK stimulation evoke any unitary current steps (Fig. 4*a*, *b*) although the usual type of currents were readily seen after excision of the patch and exposure of the physiological inside of the membrane to the high Ca concentration of the bath fluid (Fig. 4*c*, *d*). In one experiment, CCK stimulation for 4 min in the presence of antagonist did not produce any unitary current steps, but when the patch was subsequently excised the usual activity appeared. The effective CCK concentration surrounding the acinar membrane is, of course, not known in this type of experiment. The result, showing that 1.5 mM dibutyryl cyclic GMP can completely abolish the effect of CCK, indicates, however, that the CCK concentration is unlikely to have been higher than 10^{-9} M (ref. 10).

The effect of ACh stimulation was investigated in experiments with ionophoretic agonist application. In these experiments the ACh pipette could be brought close to the acinus without stimulating the cells by applying a braking (retaining) current. Effects of ejecting ACh from the pipette by ionophoresis were observed after latencies of about 10–40 s. The evoked unitary currents were indistinguishable from those evoked by CCK and the *I–V* relationships were virtually identical (Fig. 3*c*). Subsequent current recordings from an excised patch gave the usual pattern of unitary current steps with the same single-channel conductance (Fig. 3*c*).

In three experiments on excised inside-out patches (extracellular solution on both sides), removal of Ca from the bath (nominally Ca-free solution with 1 mM EGTA) rapidly abolished unitary activity (within 10–20 s), confirming our previous finding on rat pancreas[9]. In the mouse the effect of Ca removal was rapidly (10–20 s) and virtually completely reversible on Ca readmission (300 μM). In the excised patches the apparent number of channels varied between 2 and 10 with 3 or 4 channels per patch representing a common pattern in these experiments (Fig. 4).

This study of the action of CCK and ACh at the single-channel level provides direct evidence for the hypothesis previously advanced that in several gland cells, hormones and

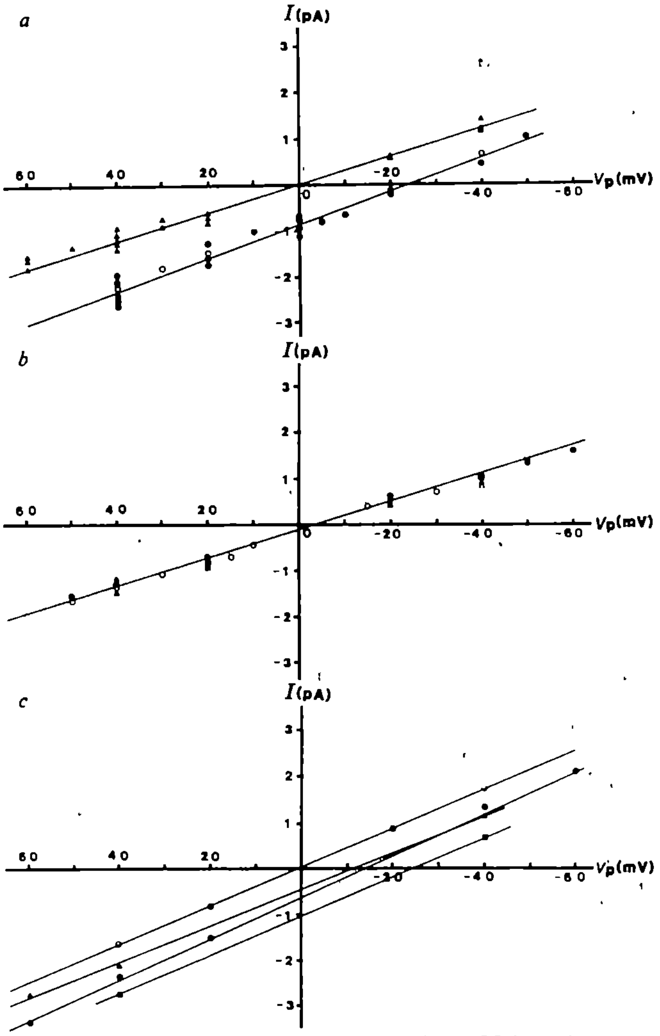

Fig. 3 Single-channel current–voltage relationships. *a*, CCK experiments. Circles represent the data from the six experiments in the *in situ* recording situation. The reversal potential corresponds to a pipette potential of about −25 mV. After excision of the patches (successful in five of the six experiments) the current–voltage relation is as represented by the triangles (symmetrical extracellular solution). The reversal potential corresponds to a pipette potential of 0. The open circles and triangles represent data from the experiment shown in Fig. 2*a–h*. *b*, Data from four excised patches (inside-out) with asymmetrical solutions. Extracellular solution in pipette and intracellular solution in bath. Reversal potential corresponds to a pipette potential of about −5 mV. *c*, ACh experiments. Filled symbols represent three individual experiments where recordings were made *in situ*. Reversal potential corresponds to a pipette potential of about −20 mV. The open circles represent data from the same experiment as the closed circles, but after excision of patch. Symmetrical saline solutions were used and the reversal potential corresponds to a pipette potential of 0.

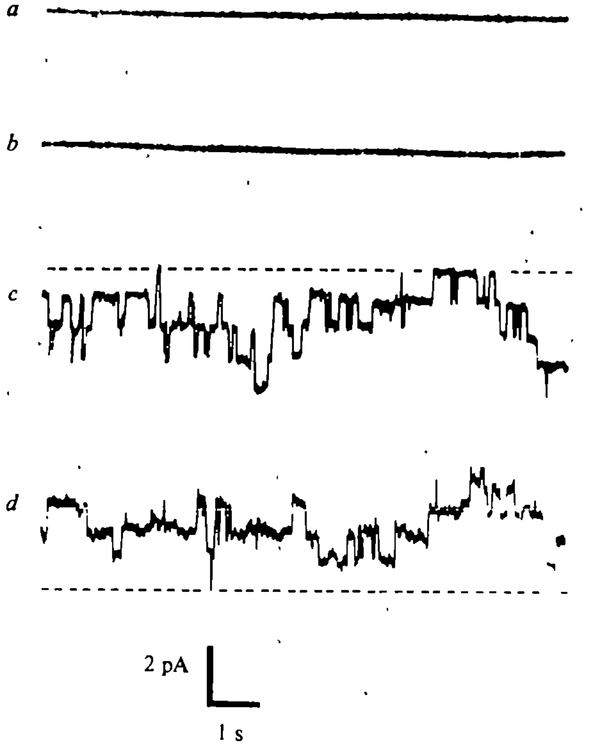

Fig. 4 The effect of CCK in the presence of 1.5 mM dibutyryl cyclic GMP in the bathing fluid. *a–d*, Consecutive traces from same patch. Pipette potential +40 mV in *a*, *b* and *c*, but −40 mV in *d*. *a*, *In situ* recording situation as shown in Fig. 1*a*, but with the CCK pipette far away from the acinus. *b*, 100 s after tip of CCK pipette has been brought close to the patch pipette as shown in Fig. 1*a*. *c*, *d*, Same patch, but after excision to expose physiological inside of membrane to extracellular bath solution having a high Ca concentration. Dashed lines represent current level when all channels are closed.

neurotransmitters control membrane permeability indirectly, via an intracellular messenger[8,17]. CCK-binding studies on pancreatic acini have revealed the existence of about 10,000 receptor sites per cell[12]. If our patches have a membrane area of about 10 μm^2 (ref. 16), we estimate that there are 100–600 channels per cell (assuming 2–10 channels per patch and one acinar cell having 600 μm^2 plasma membrane[18]). The ion transport pathway involved, a Ca-activated inward current channel, has only recently been described in cultured cardiac cells[19], neuroblastoma cells[20] and pancreatic acinar cells[9]. The experiments described here indicate that at least in the pancreatic acinar cells a hormone and a neurotransmitter can control the activation of this channel. Since CCK and ACh have been shown here to activate channels in an area (the patch area) to which these stimulants had no access, the existence of an internal messenger is postulated. Internal Ca can activate the channels and is an obvious candidate.

The mechanism for control of ion transport identified here at the single-channel level, may be the main controlling step for trans-acinar sodium and fluid transport (secretion)[17,21]. In the toad urinary bladder, current noise measurements by Lindemann and collaborators[22,23] indicate that the main hormonal (aldosterone and oxytocin) control of transepithelial Na transport (absorption) is exerted at the Na entry step by determining the number of open channels in the luminal membrane. In the pancreas, we calculate that the channels activated by CCK or ACh can account for an inward current, in the whole of an acinar unit, of up to 50 nA at a membrane potential of −30 mV, assuming an average patch area of 10 μm^2 (ref. 16), an average channel density of three per patch—the channels being open half the time[9], and using known values for the membrane surface of acinar cells and the number of cells in an electrical unit (~500)[21]. This maximal current is more than sufficient to account for the macroscopic currents actually measured in experiments with microelectrodes in which 1 μM ACh was shown to evoke an inward current of about 4 nA, accounting quantitatively for the stimulated acinar Na secretion[21].

The Ca-mediated opening of cation channels in the basolateral acinar plasma membrane evoked by CCK or ACh may represent the major control step for acinar fluid secretion in the pancreas and probably also other exocrine glands, such as the salivary glands and the lacrimal glands, where similar types of stimulant-evoked currents have been studied[17], although not at the single-channel level.

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The virus of Japanese adult T-cell leukaemia is a member of the human T-cell leukaemia virus group

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Human T-cell leukaemia virus (HTLV) has been isolated from several cases of cutaneous T-cell lymphoma in the United States[1,2]. Subsequently, adult T-cell leukaemia virus (ATLV) was also isolated from several cases of adult T-cell leukaemia in Japan[3–5]. Since the latter disease has been shown to be correlated ≥90% with the presence of high titred natural antibodies[6–8] to two structural proteins of HTLV [p24 (ref. 9) and p19 (ref. 10)], it was important to compare HTLV and ATLV. We report here that by serology of p24 and p19 and by nucleic acid homology, ATLV appears to be as closely related to the original isolate of HTLV as different isolates of HTLV (from various regions of the world) are related to each other. ATLV is therefore either identical or closely related to HTLV.

HTLV, which has recently been identified outside the United States (refs 8, 11, 12, and M.P. *et al.*, in preparation) can routinely be isolated from HTLV-positive T-cell malignancies now that mature human T cells can be cultured in liquid suspension[13–15] in the presence of T-cell growth factor[16,17]. Integrated proviral sequences have been detected in DNA of the HTLV-positive leukaemic T cells but not in DNA from normal uninfected human tissues[2,8,18,19], establishing HTLV as an acquired retrovirus of man. Moreover, sera from the HTLV-positive patients contain specific, high-titre antibodies to p24[20], the major core protein of HTLV[9] and to other HTLV structural proteins[21]. Sera from adult T-cell leukaemia (ATL) patients (from south-west Japan, where ATL is endemic)[22] also contained high-titre antibodies to HTLV structural proteins[6–8]. Independently, workers in Japan (including I.M.) described ATLV retrovirus particles in a cell line established from a patient with adult T-cell leukaemia (MT-1)[3] and in a cord blood T-cell line (MT-2) established by co-cultivation with T cells from a similar patient[4]. Serum antibodies apparently directed against this virus have also been demonstrated, both in Japanese ATL cases and in some normal people in the endemic regions[3,4]. ATLV has since been shown to possess biochemical properties typical of retroviruses[5] and is present in tumour cell but not normal cell DNA[5].

Characterization of p24[9], p19[10], reverse transcriptase[23] and nucleic acids[2,18] of HTLV shows that it is not closely related to any animal retroviruses other than having some distant similarities of amino acid sequence to p24 of bovine leukaemia virus[24]. The experiments reported here were designed to determine whether, as suspected, ATLV is the same virus as HTLV. Indirect immunofluorescence (IF) using a monoclonal antibody to HTLV p19[10], competitive radioimmunoassays utilizing homogeneously purified HTLV p24[9], and nucleic acid assays with HTLV ^{3}H-cDNA and ATLV ^{32}P-cDNA for viral mRNA and proviral DNA[2,5,18] were used to ascertain the relatedness of ATLV to previously described strains of HTLV.

DNA was prepared from the nuclear fraction of MT-1 cells and assayed for the presence of HTLV-related proviral DNA, using highly labelled ^{3}H-cDNA prepared from melittin-permeabilized[25] HTLV virus and using DNase-digested calf thymus DNA as a random primer for the endogenous reverse transcriptase. DNA from cells producing HTLV (clone B2) hybridized with HTLV cDNA with a $C_0t_{1/2}$ value of ~600 (Fig. 1), commensurate with a copy number of 3–4 per haploid genome and consistent with earlier data[18,19]. DNA from HL-60, a human myeloid leukaemia cell line[26], does not hybridize detectably to HTLV cDNA, also in keeping with earlier results with DNA from many different human and animal cell lines and fresh tissues not producing HTLV[18]. In contrast, DNA from MT-1 hybridizes extensively to HTLV cDNA. At the highest C_0t value tested (38,000), the per cent hybridization had not reached an apparent plateau (Fig. 1*a*), but was already 80% of the maximum value observed with clone B2 DNA. Due to the lengthy incubation period required, we did not test higher C_0t values. The high $C_0t_{1/2}$ of the reaction with MT-1 DNA (~1.7×10^{4}) is indicative of a copy number sufficiently low (one per eight or nine haploid genomes) to suggest that less than every cell in this line is infected. This is in keeping with earlier data on viral protein expression[3]. The high degree of homology to HTLV indicates that the detected provirus is

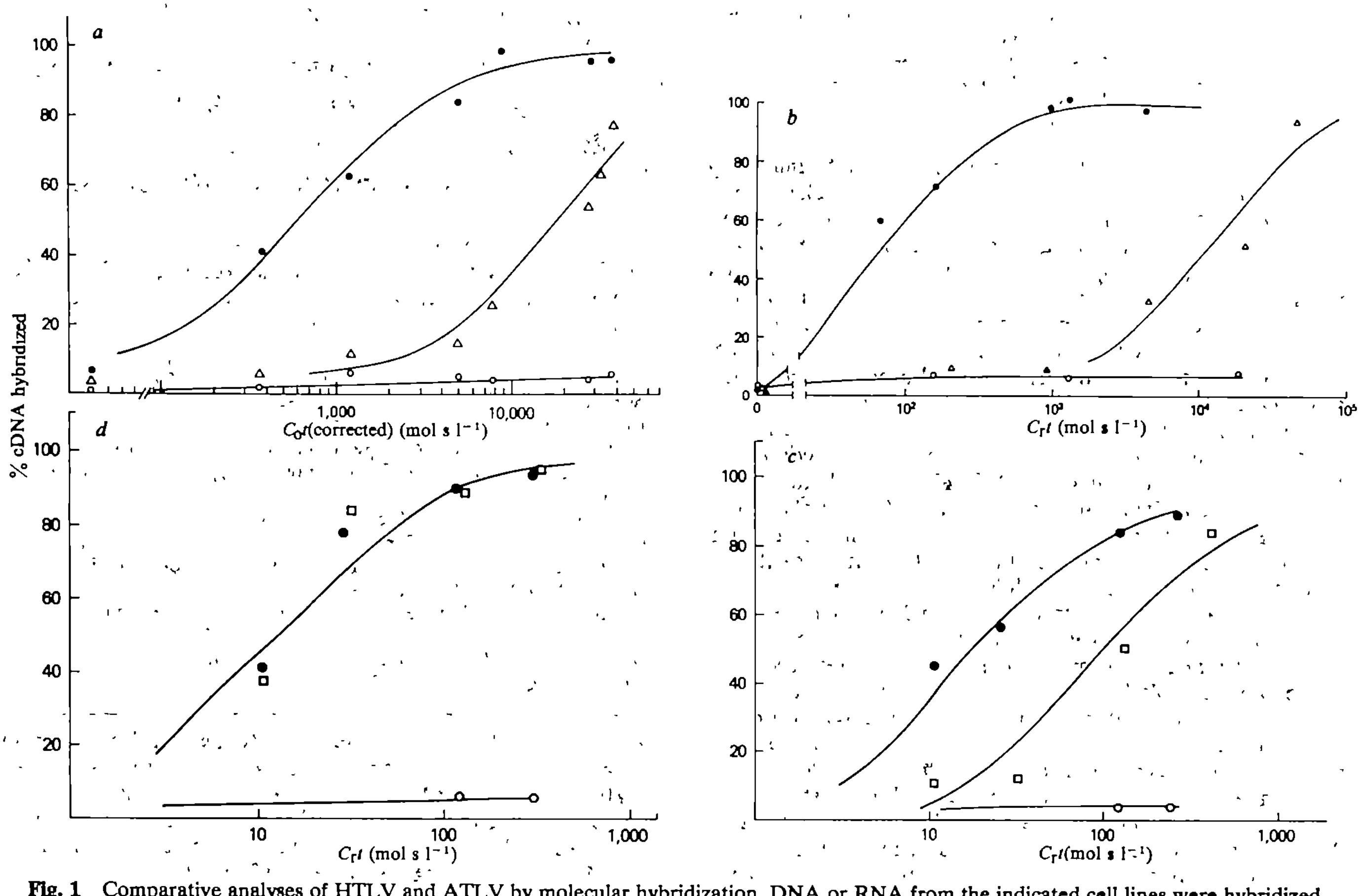

Fig. 1 Comparative analyses of HTLV and ATLV by molecular hybridization. DNA or RNA from the indicated cell lines were hybridized with HTLV ^{3}H-cDNA (*a–c*) or ATLV ^{32}P-cDNA (*d*) to the indicated C_0t (corrected) or C_rt and assayed for hybrid formation by digestion with S$_1$ nuclease as described previously[18]. Each point was determined with 500–1,000 c.p.m. cDNA and was corrected for the zero time point (5–8%) and normalized so that the homologous value = 100% (actual value 45–60%). DNA or RNA were from: clone B2 (●); MT-1 (△), MT-2 (□) and HL-60 (*a*) or CR-B cells (○) (*b–d*). *a*, ^{3}H-HTLV cDNA was hybridized to the indicated DNA (600 µg per point in 25 µl). *b*, ^{3}H-HTLV cDNA was hybridized to the indicated RNA. Clone B2 and CR-B cell RNA were poly(A)-selected and present at 25 µg per 80 µl; MT-1 RNA was unselected and present at 57 µg per 25 µl. *c*, ^{3}H-HTLV cDNA was hybridized to the indicated RNA. MT-2 RNA was not poly(A)-selected and present at 10 µg per 25 µl. *d*, ^{32}P-ATLV cDNA was hybridized to the indicated RNA.

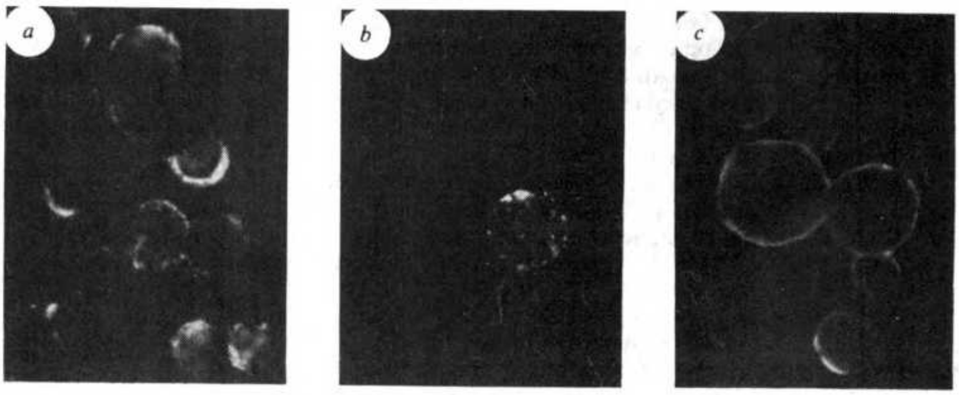

Fig. 2 Indirect immunofluorescence assay using monoclonal anti-HTLV p19. Cells were washed and fixed for 10 min with 50% methanol/50% acetone as described previously[10]. The indirect immunofluorescence assay using mouse ascites fluid containing monoclonal anti-p19 and fluorescein isothiocyanate (FITC)-conjugated F(ab')$_2$ fragment of sheep anti-mouse IgG has also been described elsewhere[10]. *a*, HUT102, clone A9 cells. *b*, MT-1 cells following treatment with 60 μg ml^{-1} IdUrd for 24 h and an additional 24 h in culture in the absence of IdUrd. *c*, MT-2 cells.

identical to HTLV or is a very closely related strain of the HTLV group. Total cytoplasmic RNA was also prepared from MT-1 cells and assayed for the presence of HTLV mRNA. At sufficient C_rt values, similar levels of hybridization are attained with MT-1 RNA and RNA from HTLV-producing cells (Fig. 1*b*), confirming that HTLV is present in MT-1 cells.

In separate experiments, HTLV and ATLV cDNA[5] were hybridized to RNA from a second cell line producing ATLV, and the values obtained were compared with those obtained with RNA from clone B2. As shown in Fig. 1*c*, HTLV cDNA hybridizes to the same extent to both RNAs, indicating that MT-2, like MT-1, contains sequences extensively related to HTLV. Moreover, ATLV cDNA also hybridizes to the same extent to both RNAs (Fig. 1*d*), confirming the close relatedness of ATLV to HTLV in reciprocal hybridization experiments. The difference in the rates of hybridization of HTLV and ATLV cDNA to MT-2 RNA (Fig. 1*c, d*) is noteworthy. HTLV ^{3}H-cDNA is randomly primed and presumably reasonably representative of the RNA genome, while ATLV ^{32}P-cDNA[5] is primarily 'strong stop' cDNA, specific to the 5′ end of the RNA (M.Y., unpublished results). The disparity in the rates of hybridization of the two cDNAs suggests that MT-2 cells express RNA transcripts which consist partly of viral leader sequences and partly of cellular sequences. This interpretation is consistent with the relatively complex pattern observed with Northern blots of MT-2 RNA using ATLV cDNA[5], where several RNA species are evident in addition to the two viral mRNAs observed with MT-1 RNA.

MT-1 cells were assayed for the presence of p19 by indirect immunofluorescence using a highly specific monoclonal antibody[10]. Anti-p19 antibody specifically labelled all six cell lines previously shown to contain HTLV (not shown), as determined by virus isolation, detection of HTLV nucleic acids and detection of antibodies to HTLV in serum from the patient. A battery of HTLV-negative cell lines (a total of 127) which included human normal and neoplastic T and B cells and several human and animal cell lines infected with or producing different animal retroviruses, were always negative. HTLV p19 was detected in a small number (~1%) of MT-1 cells. The number of fluorescent cells increased to ~8% on treatment with iododeoxyuridine (IdUrd). These numbers, although relatively low, are highly reproducible and are consistent with both the low estimated proviral copy number mentioned above and with the results of Hinuma *et al.*[3]. Virtually all the cells in the T-2 culture express HTLV p19. The range of p19-positive cells by this assay in other HTLV-producing cell lines is 5–95%. The highest percentage of anti-p19-labelled cells (besides that with MT-2) was observed with clones B-2 and A-9, derived from the HUT102 cell line (M. Maeda, M.P. and R.G., unpublished). Two additional HTLV-producing cell lines, CTCL-3[1] and CTC-16[27], established independently from the same patient (C.R.) as the HUT102 cell line, contained 20 and 38% positive cells, respectively. The proportions of fluorescent cells in CTCL-2[2] and a newly established HTLV-positive neoplastic human T-cell line, MJ (M.P. *et al.*, in preparation), were 5 and 85%, respectively.

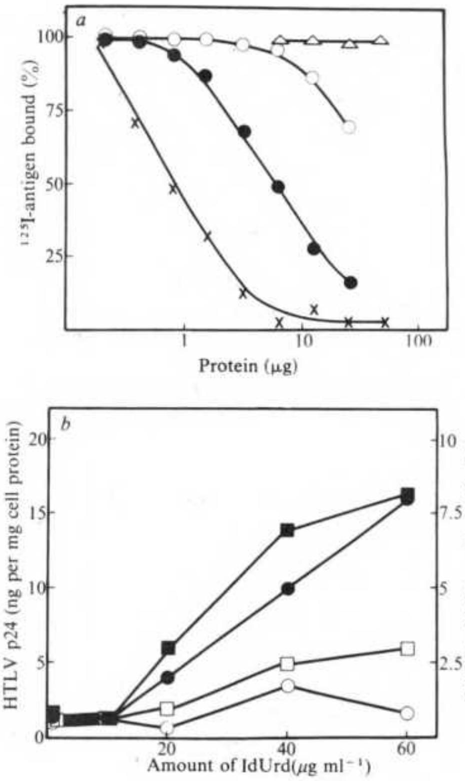

Fig. 3 Competition radioimmunoassay of HTLV p24. *a*, Homologous competition radioimmunoassay of HTLV p24. Competition radioimmunoassays were carried out with ^{125}I-labelled HTLV p24 and a limiting dilution of a rabbit antibody to HTLV. Serial dilutions (100 μl) of the solubilized cells in buffer 1 (20 mM Tris-HCl, *p*H 7.5, 200 mM NaCl, 1 mM EDTA, 0.3% Triton X-100, 0.1 mM phenylmethylsulphonyl fluoride and 2 mg ml^{-1} bovine serum albumin) were incubated with rabbit antibody to HTLV (50 μl containing 1:50 diluted normal rabbit serum) for 1 h at 37 °C. Labelled HTLV p24 (8,000 c.p.m.) in 50 μl of buffer 1 was then added and the mixture was further incubated at 37 °C for 2 h and overnight at 4 °C. A 20-fold excess of goat anti-rabbit IgG was then added and the volume was made up to 1 ml in buffer 1. The samples were further incubated at 37 °C for 1 h, 2 h at 4 °C and then centrifuged at 2,5000 r.p.m. for 5 min in a Beckman centrifuge. The supernatants were aspirated and the radioactivity in the pellet was determined in an LKB ultro-gamma counter. ×—×, Clone B2 cells; ●—●, MT-1 24 h after IdUrd induction; ○—○, uninduced MT-1 cells; △—△, cultured normal human T cells. *b*, IdUrd induction of HTLV p19 and p24 in MT-1 cells. Expression of HTLV p19 and p24 was quantified according to methods described in Figs 2 and 3 legends. Cultured MT-1 cells were exposed to different concentrations of IdUrd for 24 h and the cells were washed free of IdUrd and HTLV p19 and p24 were measured 0 and 48 h later. ○, ●, p24; □, ■, p19. Open symbols, 0 time after the 24 h IdUrd treatment; filled symbols, 48 h after removal of the IdUrd from the culture medium.

These cell lines, like CTCL-3 and CTC-16, were established from the peripheral blood of patients with leukaemia or lymoma involving relatively mature T cells. An example of the immunofluorescence obtained using monoclonal anti-p19 antibody on MT-1 and MT-2 cells and a positive control cell line, clone A9, is shown in Fig. 2.

A specific competition radioimmunoassay has been described for the HTLV core protein, p24[9], which can detect as little as 0.05 ng of p24. This precipitation assay uses ^{125}I-labelled homogeneous HTLV p24 and a hyperimmune rabbit antiserum against detergent-disrupted HTLV. Competition is observed only with proteins from disrupted HTLV or HTLV-producing cells. As shown in Fig. 3*a*, cell extracts from both MT-1 and the HTLV-producing cell line, clone B-2, competed in the HTLV p24 radioimmunoprecipitation assays. The competition with IdUrd-induced MT-1 cells was about 10-fold higher than with untreated MT-1 cells, and the slope and end point of the competition curves with clone B2 and induced MT-1 were the same. The p24 determinants of HTLV and ATLV which are recognized by this antiserum are thus indistinguishable. No competition was observed with extracts from HTLV-negative control cells, which included cultured human normal mature T cells and leukaemic T-cell lines previously shown to be HTLV negative.

The kinetics of induction of expression of p19 and p24 were analysed in parallel after treatment of MT-1 cells with IdUrd for 24 h. As shown in Fig. 3*b*, the induction of both proteins was time-dependent and increased with increasing concentrations of inducer. There was a slight increase in the expression of both p19 and p24 immediately following the 24-h treatment of MT-1 cells with IdUrd. However, a substantial increase in the expression of both proteins was observed 48 h after IdUrd treatment. At the highest IdUrd concentration tested (60 μg ml^{-1}), the p19 expression increased about 8-fold while a 16-fold increase was detected for p24 compared with the untreated control cells. The expression of both these proteins therefore increased simultaneously on IdUrd treatment of MT-1 cells. It is therefore likely that the 'adult T-cell leukaemia-associated antigen' or 'ATLA' in MT cells detected following induction with IdUrd and using natural antibodies in sera of Japanese ATL patients[3], represents one of these previously described HTLV proteins[9,10].

We have previously shown that >90% of tested Japanese patients with ATL also contained antibodies to HTLV p24[6-8]. A subsequent more extensive screen of a large number of sera of Japanese ATL patients has substantiated the earlier finding[28]. In addition, 25% of close relatives of ATL patients and about 10% of individuals in the area endemic for ATL also have antibodies to HTLV p24. In contrast, none of 40 normal random sera from the non-endemic regions of Japan had anti-HTLV p24 antibodies.

We conclude from the data presented here that the antigens reacting with the antibodies in the sera of most Japanese ATL patients are highly cross-reactive with HTLV proteins p19 and p24. Two cell lines from such patients (MT-1 and MT-2), which contain ATLV RNA and proviral DNA[5], also contain viral nucleotide sequences which are virtually indistinguishable from those of HTLV. MT-1 (which produces ATLV[3]) expresses two antigenic determinants indistinguishable from those of HTLV p24 and p19. Virtually all MT-2 cells (which produce ATLV[4]) express HTLV p19. Moreover, three other Japanese ATL cell lines established in our laboratory (M.P. *et al.*, in preparation) and two other similar lines established in Japan (S. Morikawa, personal communication) also express antigenic determinants indistinguishable from those of HTLV p24 and p19. The widespread occurrence of antibodies in the Japanese ATL sera strongly reactive with HTLV p24 further strengthens our conclusion that the retrovirus associated with Japanese ATL is identical or very closely related to previously described strains of HTLV[1,2,18] which have been isolated and characterized from patients in the US with leukaemias and lymphomas involving mature T cells, and establish ATLV within the HTLV group.

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Human T-cell clones recognize chemically synthesized peptides of influenza haemagglutinin

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The continual variation in the antigenic structure of the haemagglutinin (HA) molecule is associated with the appearance of new epidemics or pandemics of influenza in immune populations[1]. The detailed analysis of HA structure has provided the molecular basis of this phenomenon[2-4] and while four antibody-binding sites have been suggested[4], the epitopes of HA that are recognized by T cells remain undefined. Using chemically synthesized peptides corresponding to sequences of the HA molecule[5], we have investigated the antigenic determinants recognized by long-term T-lymphocyte lines and clones induced with HA and maintained in T-cell growth factor (TCGF or interleukin-2, IL-2). The data suggest that HA-specific T-cell populations contain the repertoire to respond to all the synthetic peptides analysed. However, when responses are analysed at the clonal level, one peptide located at the carboxy-terminus of the HA1 molecule and discrete from the proposed antibody-binding sites[4] seems to be immunodominant.

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Peripheral blood lymphocytes (PBL) from adult volunteers selected for known responses to strain A influenza virus (A/Texas/1/77; H_3N_2) were cultured with HA isolated from this subtype. After 6 days, cultures were either cloned by limiting dilution in the presence of TCGF, irradiated autologous PBL and specific antigen as previously described[6], or maintained as cell lines. The T lymphocyte clones and lines were maintained in continuous culture for 8 weeks and then assayed for responsiveness to specific antigen in the presence of antigen-presenting cells as determined by tritiated thymidine (^{3}H-TdR) incorporation.

Normal PBL responded to the intact virus and to the HA molecule of A/Texas/1/77, as did long-term influenza and HA lines induced with the same viral subtype (Table 1). The antigenic specificity of these lines was confirmed by their failure to respond to the unrelated strain B influenza virus, B/Singapore/222/79. Both normal PBL and T-cell lines in the presence of antigen-presenting cells responded to each of the synthetic peptides (Fig. 1) tested over a dose range of 0.01–10 μg ml^{-1}. Although these peptides were synthesized according to the sequences of influenza strain X-47 (ref. 7), in most cases they were identical to corresponding regions of A/Texas/1/77 (ref. 8). Both viruses are H3N2, and comparison of their sequences reveals only a few amino acid differences. It is evident that peptide 20, which corresponds to the carboxy-terminus of HA1, induced the maximum proliferative response (Table 1). Interestingly, this peptide is distant from the four proposed antibody-binding sites. Although these sites are able to induce proliferation, it appears that they are not the immunodominant region of the HA molecule for T-cell responses. In comparison, studies on immune responses to myoglobin in mice have demonstrated that the five antigenic sites recognized by antibody are also the primary inducers of T-cell proliferation in cell populations[9,10] and long-term cultures[11]. Furthermore, it was reported that peptides representing B-cell non-antigenic parts of the molecule were unable to induce proliferation[10], which may reflect the fact that regions of myoglobin are phylogenetically conserved and are thus immunologically silent. However, our findings demonstrate that the entire HA molecule as defined by the synthetic peptides used in these studies can induce T-cell proliferation with none of the peptides behaving as nonimmunogens, as was observed in myoglobin[10], or actively inducing suppression, as has been reported for lysozyme[12,13]. Analysis of the activity of smaller peptides of HA, however, may allow such regions to be identified.

Table 1 Reaction of normal peripheral blood lymphocytes and influenza A virus- and HA-specific long-term T-cell lines with synthetic peptides of the HA1 molecule

Synthetic peptides of HA-1 molecule	Normal PBL	Lymphocytes Influenza A line	HA line
2	2,388 (206)	10,308 (1,737)	1,797 (326)
4	3,461 (171)	1,008 (387)	2,216 (321)
7	6,204 (494)	7,885 (515)	1,497 (296)
10	3,924 (583)	7,287 (1,005)	2,088 (64)
11	5,960 (926)	11,359 (2,179)	1,349 (321)
16	2,359 (639)	9,173 (780)	1,439 (366)
17	1,667 (186)	6,683 (566)	1,470 (264)
19	2,267 (334)	10,091 (25)	1,131 (381)
20	9,061 (1,737)	30,266 (6,240)	3,549 (272)
21	2,460 (373)	5,871 (764)	1,622 (196)
22	4,733 (445)	2,065 (245)	1,378 (174)
23	3,017 (308)	8,743 (952)	2,094 (140)
HA molecule (A/Texas/1/77; H_3)	6,514 (512)	16,792 (1,808)	2,326 (452)
Intact virus (A/Texas/1/77)	16,017 (2,816)	40,645 (9,307)	9,173 (1,072)
Influenza B strain (B/Singapore/222/79)	22,085 (4,969)	57 (10)	27 (16)
Medium alone	21 (5)	20 (6)	21 (6)
TCGF	—	3,481 (365)	1,829 (52)

Normal human peripheral blood lymphocytes (PBL, 2.5×10^5 ml^{-1}) were cultured with either the synthetic peptides of the HA1 molecule, the purified HA molecule (gift of Dr R G Webster), or intact influenza A (A/Texas/1/77) or B (B/Singapore/222/79) strain virus for 6 days in RPMI-1640 (Gibco) supplemented with 10% pooled A$^+$ serum. The concentration of antigen used for the synthetic peptides and HA was 0.01–10 μg ml^{-1}, and 0.05–50 haemagglutinating units (HAU) ml^{-1} for the intact viruses. To induce T-cell lines, PBL were cultured with intact A/Texas/1/77 or the purified HA molecule of A/Texas, and after 6 days the lymphoblasts were enriched on a discontinuous Percoll gradient. These blast cells (10^5 ml^{-1}) were expanded as long-term T-cell lines with filler cells (irradiated (2,500 rad) autologous PBL at 5×10^5 ml^{-1}) and specific antigen in the presence of T-cell growth factor. TCGF was prepared by culturing PBL (1×10^6 ml^{-1}) with 0 1% purified phytohaemagglutinin-P (PHA-P; Difco) in RPMI-1640 supplemented with 1% autologous plasma[17]. After 48 h, supernatants were collected and assayed for their ability to support growth of a TCGF-dependent line as described previously[6]. The lines were maintained with filler cells and antigen every 7 days and TCGF every 3 days Influenza A virus and HA-specific T-cell lines (2.5×10^4 ml^{-1}) were cultured with the panel of antigens in the presence of irradiated autologous PBL ($1\ 25\times10^5$ ml^{-1}) for 72 h, pulsed for 8–16 h with 1.0 μCi of tritiated methylthymidine (^{3}H-TdR, NEN) and collected on to glass fibre filters. Proliferation, as correlated with ^{3}H-TdR incorporation, was measured by liquid scintillation spectroscopy[6]. Results are expressed as mean c p.m ($\pm$s.e.m.) of triplicate cultures. Only the highest proliferative response in the antigen dose–response curve is shown As controls, the responses to medium alone, TCGF and B/Singapore were determined.

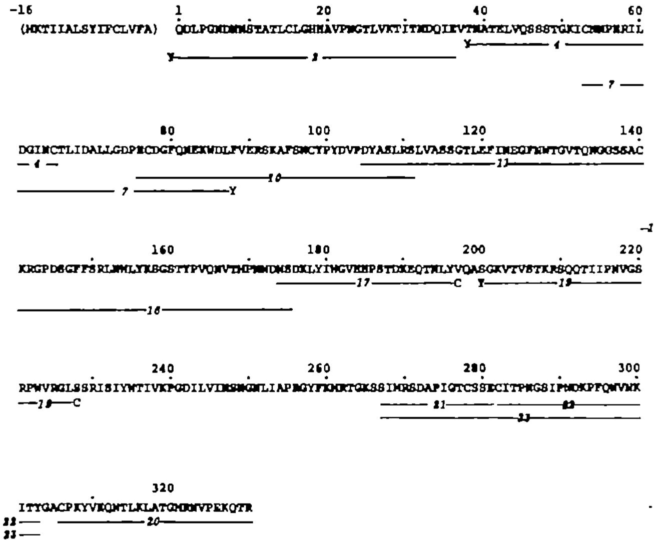

Fig. 1 Amino acid sequence of the HA1 molecule of X-47 as predicted from the nucleotide sequence[7]. The regions of the molecule synthesized are underlined and numbered. C and Y at the end of the peptide indicate, respectively, the addition of a cysteine or tyrosine not present in the primary sequence.

The fine specificity of antigen recognition by HA-specific T cells was investigated at the clonal level using chemically synthesized peptides of HA1 (Table 2). One of the four T-lymphocyte clones (HA1.9) proliferated in response to the HA molecule and the intact influenza A virus, but failed to respond to B/Singapore or to the panel of synthetic peptides. Presumably, this clone recognizes determinants that reside in the sequences not covered by overlapping peptides (Fig. 1). The remaining three clones analysed (HA1.4, HA1.7 and HA2.43) responded only to peptide 20 and not to the predominant antibody-binding sites. Comparable to this observation is a report on the clonal analysis of T-cell recognition of the antigenic determinants of myoglobin, where two clones specific for the fragment 1–55 failed to respond to peptide 15–22, which contains the only antibody-binding site[11].

To define the epitope specificity of T-cell antigen recognition, clones HA1.4, HA1.7 and HA2.43 were cultured with the intact viruses A/Japan/305/57 (H2N2) and A/Texas/1/77 (H3N2). Neither clone HA1.4 nor HA1.7 responded to A/Japan, but both proliferated in response to stimulation with A/Texas (Table 3), suggesting that these two clones recognize a variant region of the carboxy-terminus of HA1. Any one of the amino acid substitutions between A/Japan and A/Texas occurring within the sequence of peptide 20 at residues 312–314, 316, 321 and 326–329 (Fig. 2) would be able to confer the specificity of these clones for A/Texas as opposed to A/Japan, as it has been reported that T-cell activation may

Table 2 Recognition of synthetic peptides by HA-specific human T-lymphocyte clones

Synthetic peptides of HA1 molecule	Clone no. HA1 4	HA1.7	HA1.9	HA2.43
2	61 (5)–92 (16)	17 (6)–59 (13)	25 (11)–57 (15)	17 (5)–57 (14)
4	24 (2)–54 (14)	17 (1)–31 (11)	25 (5)–37 (9)	49 (9)–81 (9)
7	33 (7)–136 (50)	13 (4)–38 (1)	16 (8)–51 (19)	10 (1)–61 (7)
10	31 (9)–96 (72)	27 (3)–38 (5)	26 (9)–52 (14)	28 (4)–74 (9)
11	15 (3)–47 (5)	19 (5)–81 (25)	14 (3)–37 (1)	17 (4)–37 (9)
16	16 (4)–65 (45)	15 (4)–26 (10)	14 (8)–93 (51)	15 (4)–43 (8)
17	24 (5)–40 (8)	20 (5)–45 (23)	20 (5)–45 (23)	18 (7)–19 (2)
19	27 (5)–51 (4)	27 (5)–67 (14)	14 (2)–49 (12)	31 (5)–61 (14)
20	69 (46)–3,591 (22)	25 (4)–2,846 (158)	33 (15)–83 (23)	886 (30)–13,662 (1,514)
21	21 (2)–48 (2)	22 (2)–44 (15)	16 (4)–43 (7)	17 (2)–31 (6)
22	18 (4)–44 (7)	19 (4)–39 (7)	16 (2)–28 (4)	11 (3)–21 (3)
23	12 (1)–22 (3)	48 (9)–63 (6)	21 (3)–52 (16)	9 (2)–39 (3)
HA molecule (A/Texas/1/77, H_3)	2,607 (282)	3,073 (180)	10,440 (566)	12,038 (280)
Intact virus (A/Texas/1/77)	7,772 (732)	3,009 (99)	22,889 (6,751)	14,401 (926)
Influenza B strain (B/Singapore/222/79)	37 (11)	29 (2)	43 (6)	18 (2)
Medium alone	20 (1)	27 (4)	27 (4)	29 (2)
TCGF	3,813 (366)	1,018 (29)	8,099 (521)	1,276 (25)

HA-specific human T-lymphocyte clones were isolated as described previously for influenza A virus[6]. Briefly PBL were cultured for 6 days with 0.1 μg ml^{-1} HA. The lymphoblasts enriched on a discontinuous Percoll gradient and resuspended at $33\frac{1}{3}$ cells ml^{-1} in RPMI–1640 supplemented with 10% A^+ serum and 20% TCGF, were plated out at one cell every third well in Terasaki trays with 10^4 irradiated autologous PBL and 0.1 μg ml^{-1} HA. After 7 days, growing clones were transferred to 96-well microtitre trays and then to 24-well trays Then they were expanded in 25-cm^2 tissue culture flasks. At each transfer the T-lymphocyte clones received fresh TCGF and irradiated autologous PBL, together with specific antigen. The clones were maintained with 20% TCGF every 3–4 days and irradiated autologous PBL and HA were added every 7 days. Before use in proliferation assays, the clones were allowed to rest for 6–7 days after the addition of filler cells. HA-specific clones (2.5×10^4 ml^{-1}) were cultured with the synthetic peptides of HA1 molecule (0.01–10 μg ml^{-1}), HA molecule (0.01–10 μg ml^{-1}), A/Texas/1/77 (0.05–50 HAU ml^{-1}) or B/Singapore/222/79 (0.05–50 HAU ml^{-1}) in the presence of irradiated autologous sheep erythrocyte rosette-negative (E^-) cells (2.5×10^4 ml^{-1}) for 72 h, and proliferation measured by ^{3}H-TdR incorporation. E^- cells were fractionated as described previously[6]. Results are expressed as mean c.p.m. (±s.e.m.) of triplicate cultures. Both the lowest and highest proliferative responses for the antigen concentrations used are shown for the synthetic peptides.

Table 3 Specificity of T-cell clones for A/Japan/305/57 (H2N2) and A/Texas/1/77 (H3N2) subtypes of influenza A virus

Antigen	Clone no. HA1 4	HA1 7	HA2.43
Peptide 20	1,145 (138)–15,265 (1,356)	1,047 (207)–17,408 (1,799)	620 (65)–3,373 (481)
A/Texas/1/77	645 (134)–15,561 (438)	975 (251)–17,406 (822)	472 (109)–3,089 (396)
A/Japan/305/57	55 (29)–91 (21)	63 (49)–123 (16)	274 (28)–2,620 (229)
B/Singapore/222/79	19 (5)–42 (6)	25 (3)–56 (2)	27 (3)–49 (9)
Medium alone	21 (3)	55 (23)	11 (3)
TCGF	1,409 (24)	1,982 (57)	1,472 (139)

HA-specific clones (2.5×10^4 ml^{-1}) were cultured with peptide 20 (0.01–10 μg ml^{-1}), A/Texas/1/77, A/Japan/305/57 or B/Singapore/222/79 in the presence of irradiated autologous E^- cells (2.5×10^4 ml^{-1}) for 72 h. The intact influenza viruses were added at concentrations of 0 05–50 HAU ml^{-1}. Proliferation was determined by the incorporation of ^{3}H-TdR Results are expressed as described in Table 2 legend.

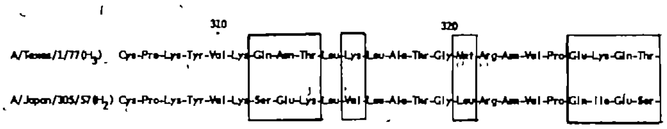
310
320
A/Texas/1/77 (H3)
Cys-Pro-Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr-Gly-Met-Arg-Asn-Val-Pro-Glu-Lys-Gln-Thr-
A/Japan/305/57 (H2)
Cys-Pro-Lys-Tyr-Val-Lys-Ser-Glu-Lys-Leu-Val-Leu-Ala-Thr-Gly-Leu-Arg-Asn-Val-Pro-Gln-Ile-Glu-Ser-

Fig. 2 Amino acid substitutions between A/Japan/305/57 and A/Texas/1/77 occurring within the sequence of peptide 20. The sequence comparison was derived from Gething *et al.*[2]. The sequence numbering is according to Min Jou *et al.*[7] in order to conform with our peptide designations. The boxed areas indicate substitutions.

be sensitive to a single amino acid substitution[14,15]. In contrast to clones HA1.4 and HA1.7, clone HA2.43 recognized both viral subtypes, which implies that it recognizes an invariant sequence of peptide 20, or less likely that the amino acid substitutions between A/Japan and A/Texas are insufficient to cause modification of the molecule to influence T-cell antigen recognition[14].

The findings reported here do not resolve the issue of the nature of T-cell recognition in terms of a defined amino acid sequence or its dependency on tertiary structure[16]. In an attempt to define the epitope sequence, subunits of peptide 20 were generated with the four carboxy-terminal amino acids and increasing the subunit sequence progressively by four amino acids until the complete peptide was synthesized. Unlike the intact peptide, however, none of the subunits induced proliferation of the clones (data not shown). This result does not distinguish whether the amino terminus of peptide 20 is the precise sequence recognized or whether it dictates the conformation of the native peptide necessary for T-cell recognition. An alternative explanation is that the subunits themselves cannot adopt the same conformation as in the intact peptide, therefore they are no longer recognized by the T cells.

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Linkage relationship of a cloned DNA sequence on the short arm of the X chromosome to Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is one of the most common and serious human X-linked disorders. It occurs at a frequency of up to 1 in 5,000 newborn males in most populations studied, with about one-third of all cases due to new mutations[1]. The primary biochemical defect remains unknown, and no proven prenatal diagnostic test exists, although raised serum creatine kinase levels act as a somewhat equivocal guide to carrier females[2]. Previous studies have shown no measurable genetic linkage of the *DMD* locus with any X-chromosome marker[3,4]. Therefore, if a cloned sequence of the X chromosome could be used to define the locus, and to provide a closely linked set of markers, it would be of considerable importance in the prediction and prevention of DMD, as well as a step towards identifying the basic biochemical defect causing the disease. We present here evidence of an X-chromosome sequence, defined by its restriction enzyme polymorphism, that is loosely linked to *DMD*, at a distance of approximately 10 centimorgans, as determined by studies on nine informative families. The polymorphism occurs in 29% of women in a control London population and in 22% of carriers for *DMD*. The linkage data support cytogenetic evidence that *DMD* is on the short arm of the X chromosome. The object of this letter is to encourage others to make use of our probe, which seems to be linked to the *DMD* locus.

Restriction fragment length variation results from changes in the primary DNA sequence, such as single base changes introducing or deleting a restriction site, or sequence deletions, additions or translocations affecting the length of DNA between sites. These DNA sequence polymorphisms are inherited in a mendelian fashion and may be used in conventional linkage studies[5–8]. Variations in restriction sites around the human β-globin genes have been estimated to occur once in every 100 base pairs[9]. Polymorphisms are sufficiently frequent in the human genome to assume that one will be found adjacent to any random cloned DNA probe several kilobases (kb) in length. A set of such sequences can be used to construct a linkage map of the entire human genome, or of a particular chromosomal region[6,10–13].

We have previously cloned a collection of sequences from the human X chromosome in λ phage after sorting using a cytofluorimeter; approximately 50,000 clones were obtained, sufficient to ensure that >90% of sequences from the X chromosome are represented in the library[14].

Single-copy sequences were identified in the X-chromosome library using Benton–Davis screening[15] to total labelled human DNA; clones containing repetitive elements present at >100 copies in the genome hybridize and are labelled in the reannealing conditions used[16]. One of the single-copy clones obtained, given the laboratory acronym λRC8, was characterized by hybridization to *Eco*RI-digested human DNA, DNA from various rodent–human hybrid cell lines containing X chromosomes or fragments as the only human genome component, and mouse DNA using Southern blots[17].

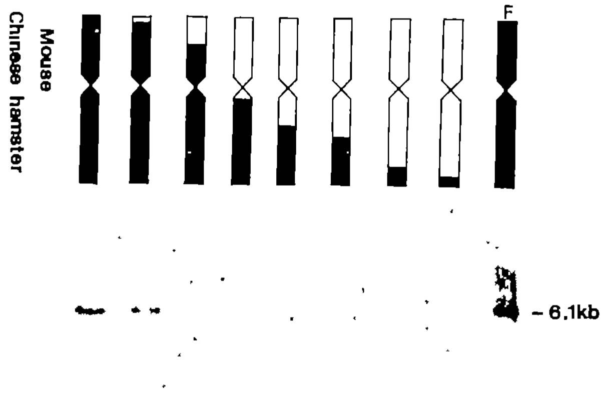

Fig. 1 Localization of λRC8 to the region Xp21–Xp223 using *Eco*RI-digested DNA from a panel of rodent–human cell hybrids. Positioned above each lane is a representation of the human X chromosome, shaded to show the portion which is present in the hybrid cell line from which the DNA was obtained (from left to right): Chinese hamster parental cell line, mouse parental cell line; hybrids containing part or all of the human X chromosome: Xpter–Xqter, Xp223–Xqter, Xp21–Xqter, Xq12–Xqter, Xq21–Xqter, Xq22–Xqter, Xq26–Xqter, Xq28–Xqter, fibroblast cell line (F). These cell lines were characterized cytogenetically and by isozyme markers in the department of Genetics, University of Freiburg. High molecular weight total DNA was extracted, digested and analysed by Southern blotting as previously described[23,24]. Blots were washed in 1×SSC at 65 °C and autoradiographed at −70 °C for 72 h.

This clone contains a 6.1-kb insert of human DNA and hybridizes to a single 6.1-kb band in *Eco*RI-digested human genomic DNA, and DNA from HORL9.X cells (a mouse–human hybrid cell line in which the only human chromosome is the X chromosome), but not to mouse DNA (Fig. 1). It hybridizes to 46XY, 46XX and 48XXXX genomic DNAs with an intensity relative to the number of X chromosomes present (not shown), demonstrating that it is X-chromosome specific. In hybridizations to total human DNA it gives a slight background after moderate stringency washes (1×SSC) which disappears after washing filters at a higher stringency (0.1×SSC) and does not interfere with the analysis reported here.

Somatic cell hybrids were used to locate the DNA sequence cloned in λRC8 to a region of the X chromosome. Hybridization of the clone to DNA samples from various rodent–human cell lines containing portions of the human X chromosome localized λRC8 to the region Xp21–Xp223 (Fig. 1). There was no correlation between hybridization and the spectrum of autosomes present in these cell lines.

Table 1 Linkage data for Duchenne muscular dystrophy, probe λRC8, Xg blood group and colour blindness (CB)

θ	DMD–λRC8 $n = 9$	DMD–Xg $n = 10$	DMD–CB $n = 3$	λRC8–Xg $n = 4$
0 01	+0.393	−13.403	−9.781	−0.711
0.05	+1.535	−6.667	−5.018	−0.099
0.10	+1.766	−3 986	−3.082	+0.090
0 15	+1.715	−2.573	−2.028	+0.050
0.20	+1.543	−1.683	−1.343	+0.161
0.25	+1.305	−1.085	−0.865	+0.144
0 30	+1.033	−0.675	−0.524	+0.115
0.35	+0.753	−0 394	−0.283	+0.078
0.40	+0 481	−0.203	−0.123	+0.046
0 45	+0.229	−0.078	−0 030	+0.017

Results are expressed as lod scores (log of the odds) for different values of recombination fraction (θ) The number of kindreds informative for each comparison is indicated (n).

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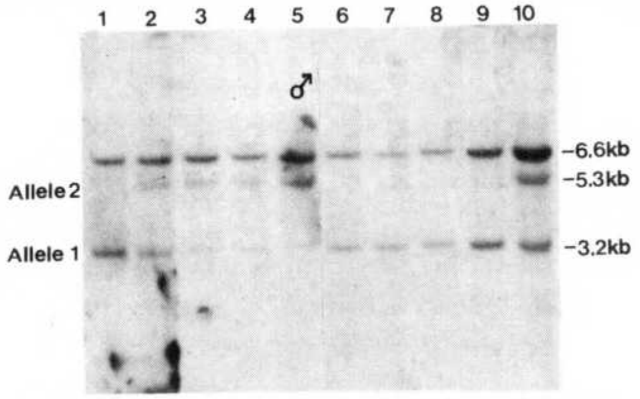

Fig. 2 Variants identified by λRC8 in *Taq*I-digested total DNA from obligatory carriers of DMD. Sample 5 is from the affected son of obligatory carrier 2. Heterozygotes have bands at 5.3 and 3.2 kb; homozygotes have either the 5.3- or 3.2-kb band. Blood samples (10 ml) were taken from Duchenne patients, carriers and normal controls and total DNA prepared[24]. *Taq*I-digested DNA (10 μg) was analysed by Southern blotting[17,23]. After washing to moderate stringency (1×SSC), filters were autoradiographed using Fuji X-ray film and intensifying screen for 72 h at −70 °C.

λRC8 was used to identify polymorphisms by screening panels of normal female DNAs digested with different restriction enzymes. It was found to give a variable pattern of bands with *Taq*I-digested total human DNA, hybridizing to the following fragments: an invariant band of 6.6 kb and a second fragment of either 5.3 or 3.2 kb (Fig. 2). The cloned sequence did not identify polymorphisms with 15 human DNAs digested with *Msp*I, *Xba*I, *Bam*HI or *Sst*I.

Females showing hybridization to both 5.3- and 3.2-kb bands are heterozygous for the restriction fragment length polymorphism: they have one X chromosome which gives a 6.6-kb plus a 3.2-kb fragment (pattern 1), and a homologous X chromosome where the *Taq*I site is altered and the probe hybridizes to give fragments of 6.6 kb plus 5.3 kb (pattern 2). In *Taq*I digests of male DNA, λRC8 only hybridizes to two bands, the 6.6-kb fragment and either the 3.2-kb or the 5.3-kb fragment, never to three fragments. The nature of the polymorphic change has not been investigated but is irrelevant to the linkage analysis. It is only necessary that the alteration is stable and is inherited in a mendelian fashion.

Of a total of 26 *Taq*I-digested normal female and 23 normal male DNAs screened, 7 females were heterozygous (2/1) and 3 males had the rarer allele (2). All the other samples had the common allele (1). Calculated from the males, this gives a gene frequency for the rarer allele of 0.13 and is consistent with the *Taq*I polymorphism identified by λRC8 behaving as a single-copy X-chromosome sequence.

Families in which DMD occurred in at least two generations, and preferably with a living maternal grandfather, were identified from a register of DMD families in Wales, supplemented by families from other centres. Only those families in which the diagnosis was fully documented or which could be confirmed clinically were studied. Key obligatory carriers were first identified and blood samples taken. DNA was prepared, digested with *Taq*I and hybridized with radioactive λRC8 probe to determine which key carriers were heterozygous for the restriction fragment length polymorphism. If the key carrier was heterozygous, samples were collected from all other available informative members of the family. Sampling of female members was restricted to obligatory carriers; those females considered carriers on the sole basis of biochemical assay (serum creatine kinase) were excluded.

DNA from 45 unrelated obligatory carriers for Duchenne was digested with *Taq*I and screened with λ RC8; nine were heterozygous for the polymorphism, giving gene frequencies in the Duchenne carriers of 0.89 for allele 1 and 0.11 for allele 2. It is thus present at the same frequency in DMD families as in the control London population. The *Taq*I polymorphism was traced through nine families (Fig. 3) and is inherited in a mendelian fashion. Of 24 meioses, 21 retained linkage and 3 showed recombination.

Lod scores for the linkage between the polymorphism and the *DMD* locus (Table 1) were calculated using the LIPED computer program[18] and checked by hand. The overall probability of linkage is equivalent to the area beneath the curve of likelihood values at different genetic distances.

A lod score expresses in logarithmic form the probability that a linkage exists between two loci; for *DMD* and λRC8, the probability of linkage at a distance of ~10 centimorgans is 58:1 (making the assumption that linkage exists). The overall probability of any linkage is 22:1. The use of lod scores in this way is explained in detail by Race and Sanger[19]. From our experience, an analysis of at least 50 more obligatory carriers from well characterized DMD families would provide a further 10 informative pedigrees to confirm this assignment absolutely; therefore, we are making λRC8 available to others with access to DMD families.

DMD shows negative lod scores with colour blindness (Table 1). This is to be expected because colour blindness is within the *G6PD* linkage group on the end of the long arm of the X chromosome (Xq28)[20]. It provides a negative control and demonstrates the difference in lod scores between unlinked (but syntenic) and loosely linked loci. Using the Xg blood group, we have not yet studied sufficient informative families for λRC8 to obtain significant results, but clearly we have confirmed that there is no measurable linkage between *Xg* and *DMD*, thus confirming the exclusion of the terminal portion of the short arm of the X chromosome as the site of the *DMD* locus.

The results obtained suggest that the Duchenne locus is localized approximately in the middle of the short arm of the X chromosome. This supports data from six female cases of Duchenne muscular dystrophy with balanced *de novo* X-chromosome to autosome translocations with break points at Xp21 (refs 21, 22). As these data depend on an unequivocal clinical diagnosis of DMD in females, they have hitherto been regarded as highly suggestive rather than conclusive.

In this analysis, we have assumed that DMD is a single gene defect. As in the case of the β-globin gene locus in β-thalassaemia, it is possible that only a single locus is involved, but with several different molecular defects at that gene.

These data support the localization of the gene mutation causing Duchenne muscular dystrophy to the short arm of the X chromosome, and provide an example of how to use recombinant DNA technology to define linkage to a phenotype caused

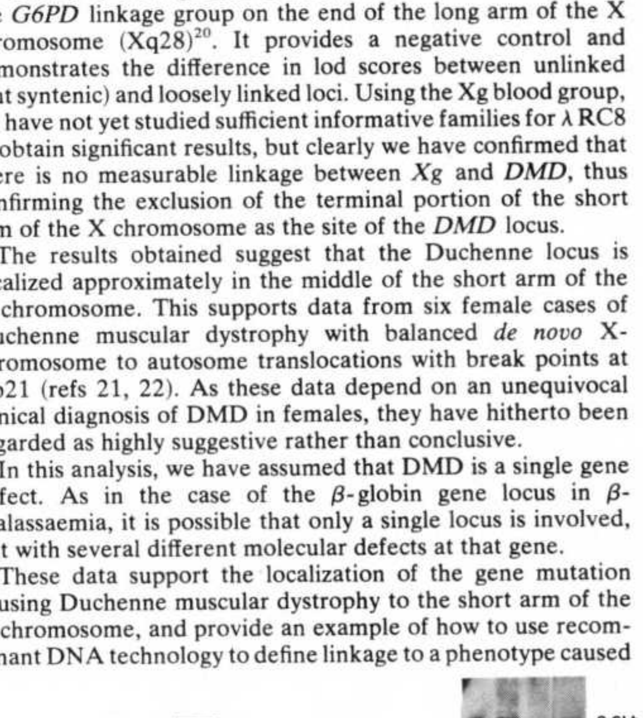

Fig. 3 Segregation of the λRC8 polymorphism through a DMD family showing the mendelian inheritance of the variant and also a cross-over between the two loci. Nine families were studied; full details are available from the authors. Symbols: square, male; black square, affected male; circle, female; dotted circle, obligatory carrier; oblique line through symbol, dead. The autoradiograph shows the hybridization of λRC8 to *Taq*I-digested DNA from members of the pedigree: *a*, grandfather; *b*, mother; *c*, *d*, sons.

by a single gene defect where the primary biochemical defect is unknown.

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Anti-ovalbumin monoclonal antibodies interact with their antigen in internal membranes of *Xenopus* oocytes

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The microinjection of selected antibodies into the cytosol or nucleus of living cells is proving a useful technique for investigating the function of intracellular proteins[1–5]. The rationale underlying this approach is that, in binding to its antigenic determinant, the antibody may mask or disrupt the activity mediated by the region of the protein near the determinant. This technique, however, cannot be used to identify functions related to antigenic determinants of proteins within the lumen of the endoplasmic reticulum (ER), or Golgi compartments, since both regions are inaccessible to direct microinjection and since the movement of proteins into these areas from the cytosol occurs during but not after their translation[6]. In theory, this constraint could be overcome if the mRNAs specifying the antibody were injected into the cell containing the target antigenic determinant. After the vectorial and co-translational transfer of its constituent chains through the membrane, the antibody would then be assembled within the lumen of the ER thereby being in a position to bind to its antigenic determinants. We demonstrate here a successful example of such an approach.

To study the interaction of antibody and antigen we made use of *Xenopus* oocytes. These cells will faithfully export secretory proteins after injection of the encoding mRNAs[7,8]. Additionally both assembly and secretion of a mouse immunoglobulin, MOPC 21, have been shown to occur normally in oocytes[9]. However, since the antigens recognized by this antibody are unknown, the biological activity of the oocyte product remains untested. Moreover, the oocyte might not possess the high levels of disulphide interchange enzyme thought to be necessary in immunoglobulin-producing cells for the production of correctly located S–S bonds within the immunoglobulin molecule[10]. In this letter we describe the synthesis in oocytes of murine monoclonal antibodies raised against chick ovalbumin, a protein whose synthesis and secretion from oocytes has been well characterized[11,12]. The antibodies synthesized by the oocyte maintain their specificity. When ovalbumin mRNA is also injected the formation of antigen–antibody complexes within the internal membranes of the oocyte results in a severe reduction in secreted antibody, even when the ovalbumin mRNA was introduced 24 h later.

Spleen cells from a BALB/c mouse immunized with ovalbumin were fused to P3-NS1 myeloma cells in the presence of polyethylene glycol[13]. The fusion was screened by an indirect trace binding assay[14] and positive wells cloned by the dilution method[15]. Two cloned hybridomas 7/2 and 3/4, both IgG_1, were chosen for the microinjection studies. Binding studies carried out under near-saturating conditions for both monoclonals and detecting antibody suggested that their binding was additive, indicating that they recognized different antigenic determinants on the ovalbumin molecule (data not shown). Furthermore, the stoichiometry suggested single antigenic sites on the molecule.

Immunoprecipitates of the immunoglobulin chains synthesized by the hybridoma cells had the same electrophoretic mobility as the analogous immunoprecipitated products made in oocytes after injection of the hybridoma mRNAs (Fig. 1*a*). Chick ovalbumin made by oocytes differs slightly in its post-translational modification from the oviduct protein[11]. Nevertheless the immunoglobulins made by the hybridoma cells bind to ovalbumin synthesized by oocytes after injection of oviduct mRNA (Fig. 1*b*).

Since the oligosaccharide moiety elaborated by oocytes for the glycosylation of immunoglobulins (A.C. and G.V., unpublished data) and other foreign proteins[16] differs from the 'native' side-chain, it was possible that the immunoglobulins synthesized by the oocyte would lack biological specificity. However, the monoclonal antibodies translated by the oocytes bound specifically to an ovalbumin immunoabsorbant (Fig. 1*c*).

The data above show that the monoclonal antibodies translated by oocytes and subsequently extracted will bind to ovalbumin. However, antibody conformation is dependent on correct disulphide bond formation. Since the exact redox potential within the ER of cells is unknown, and since disulphide bonds in immunoglobulins might be somewhat labile within the ER due to the presence of a disulphide-exchange enzyme[10], the biological activity manifested by the immunoglobulins after their extraction from oocytes might not exist in the intracellular environment. In order to examine this possibility we have injected various combinations of oviduct and hybridoma mRNAs into oocytes. The oocytes were incubated in medium containing ^{35}S-methionine for 24 h before oocytes and media were collected for analysis. Oocyte homogenates and media were immunoprecipitated with rabbit anti-chick ovalbumin or goat anti-mouse immunoglobulin antibodies, and the resulting immunoprecipitates examined by polyacrylamide gel electrophoresis (Fig. 2).

When the oocytes were injected with ovalbumin mRNA and both monoclonal mRNAs, the monoclonal antibody chains could be specifically precipitated by rabbit anti-ovalbumin (Fig. 2, track 3). Similarly, from the same oocytes, ovalbumin could be specifically precipitated by goat anti-mouse immunoglobulin (Fig. 2*b*, tracks 3 and 9). However, if only one monoclonal mRNA was co-injected with ovalbumin mRNA, this cross-precipitation effect was never observed although some non-

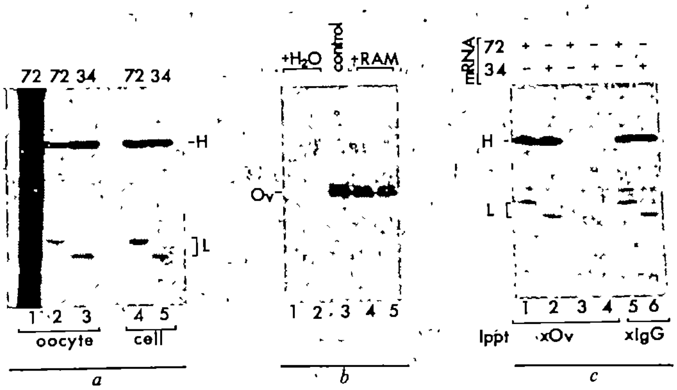

Fig. 1 Biological specificity of antibodies made by oocytes The 3/4 and 7/2 lines were grown in Dulbecco's modified Eagle's medium containing 10% horse serum (Difco) supplemented with hypoxanthine, aminopterin and tymidine Cells were labelled with ^{35}S-methionine (Amersham) as described by Cotton *et al.*[22] Media and cell homogenates were retained for immunoprecipitation and electrophoresis. Hybridoma mRNA was prepared by lysing 5×10^9 cells in hypotonic buffer containing 0 1% diethylpyrocarbonate (Sigma). The post-nuclear supernatant was twice phenol-extracted and then ethanol precipitated The RNA pellet was resuspended in 5 ml of 10 mM Tris-HCl *p*H 7.5, 1 mM EDTA, 0.1% SDS and 0.5 M LiCl, bound to oligo (dT) cellulose (Type III, Collaborative Research) and eluted with the same buffer minus LiCl. The polyadenylated mRNA was then ethanol precipitated and resuspended in distilled water at 1 mg ml^{-1}. Polyadenylated oviduct mRNA was prepared from laying Rhode Island Red hens as described by Palmiter[23]. For clarity this mRNA is referred to as ovalbumin mRNA in the text Oocytes were microinjected with 30 nl of mRNA and cultured in Barths saline (5 oocytes/30 µl) containing antibiotics, 6% fetal calf serum and 1 µCi µl^{-1} ^{35}S-methionine (300 Ci mmol^{-1}) for 24 h at 21 °C. Culture media were retained for electrophoresis whilst oocytes were homogenized in 100 mM NaCl, 1% Nonidet P40, 1 mM phenylmethylsulphonyl fluoride, 25 mM Tris *p*H 7.6 as previously described[7]. For electrophoresis the cell lysates, oocyte fractions and culture media were immunoprecipitated (see below) Precipitates were dissolved in sample buffer (20 mM Tris-HCl *p*H 9.2, 20% glycerol, 1 mM phenylmethylsulphonyl fluoride, 1% β-mercaptoethanol, 0 01% bromophenol blue and 2% SDS) and 25 µl aliquots were run on 12.5% polyacrylamide gels, followed by fluorography[24] Numbers above tracks refer to mRNA injected (oocyte) or cell lines used (cells). *a*, Synthesis and secretion of immunoglobulins by cells and oocytes. 50 µl aliquots of homogenates from mRNA-injected oocytes (tracks 2, 3) or labelled cells (tracks 4 and 5) were immunoprecipitated with 3 µl of goat anti-mouse IgG (Miles) using *Staphylococcus aureus* envelopes as previously described[9] Track 1 contains 5 µl of oocyte homogenate before immunoprecipitation. *b*, Ovalbumin made by oocytes binds to hybridoma antibodies Oocytes were injected with ovalbumin mRNA, labelled with ^{35}S-methionine and homogenized as described above. 50 µl of homogenate were mixed with 500 µl of immunoprecipitation buffer[9] and with 50 µl of hybridoma cell medium containing the monoclonal antibodies. The above mixture was incubated for 1 h on ice. Then either 10 µl of rabbit anti-mouse IgG (RAM) (tracks 4 and 5) or 10 µl of distilled water (tracks 1 and 2) were added and the incubation was continued for another 30 min on ice. Finally 50 µl of *S. aureus* suspension were added. The immunoprecipitation was completed as usual and samples run into a gel as described above and fluorographed. Track 1, 3.4 medium without RAM, track 2, 7 2 medium without RAM; track 3, control immunoprecipitated with rabbit anti-ovalbumin, track 4, 3.4 medium plus RAM, track 5, 7.2 medium plus RAM. Ov, ovalbumin. *c*, Oocyte anti-ovalbumin immunoglobulins bind to ovalbumin 50 µl aliquots of 10% *S. aureus* suspension in immunoprecipitation buffer were incubated with 3 µl of rabbit anti-ovalbumin (Miles, tracks 1–4) or with 3 µl of goat anti-mouse IgG (tracks 5, 6), for 30 min at room temperature in order to bind the antibodies to the bacterial envelopes The *S. aureus* envelopes were then spun and rinsed twice with 500 µl of immunoprecipitation buffer, resuspended in 500 µl of the same buffer and incubated for 30 min in presence (tracks 1, 2 and 5, 6) or absence (tracks 3, 4) of 0 2 mg ml^{-1} ovalbumin (Sigma) The envelopes were then rinsed twice as above and resuspended in 500 µl of immunoprecipitation buffer Meanwhile, homogenates were made from oocytes previously microinjected with 7.2 mRNA or 3.4 mRNA and subsequently labelled as in Fig. 2*a* 10 µl of oocyte homogenate were added to the appropriately prepared, resuspended envelopes and incubated overnight at 4 °C The rest of the immunoprecipitation and the electrophoresis weere carried out in the standard way described above. Ov, ovalbumin; H, immunoglobulin heavy chain, L, immunoglobulin light chain, Ippt, immunoprecipitation with rabbit anti-chick ovalbumin (xOv) or goat anti-mouse IgG (xIgG).

specific precipitation of ovalbumin occurred which disappeared if unlabelled ovalbumin was included in the homogenization medium (compare Fig. 2*b*, tracks 2–4 with tracks 8–10). This complex was not found when oocytes containing the two monoclonal antibodies and oocytes containing ovalbumin were mixed before homogenization (data not shown). We therefore interpret the co-precipitation of ovalbumin and the two monoclonal antibodies as indicating their association *in vivo* within the membraneous systems of the oocyte.

Figure 2 shows that the two monoclonal antibodies were also found associated with secreted ovalbumin present in the culture media (Fig. 2*a*, track 11; Fig. 2*b*, track 13). However, control experiments revealed that such association could take place in the media after secretion (data not shown) so it is not clear whether the monoclonal antibody–ovalbumin complex can be secreted.

The effect on secretion resulting from interactions between the immunoglobulins and ovalbumin was assessed by comparing the relative amounts of each protein inside and outside the oocyte under the different injection regimes. The results of some representative experiments are shown in Fig. 3*a*. Only ovalbumin and the two immunoglobulin γ chains were considered since excess light (k) chains are made and secreted by the oocytes (data not shown); these supernumerary chains will not be present in complexes between ovalbumin and tetrameric immunoglobulin. The results in Fig. 3*a* clearly show that not only was there a marked reduction of heavy chain secretion when both monoclonal antibodies were present with ovalbumin, but also when each immunoglobulin alone was present with ovalbumin. This latter result was surprising in view of our failure to detect any specific interactions by immunoprecipitation in these cases (see Fig. 2).

No significant reduction of ovalbumin secretion was seen or expected in these experiments, since only a small proportion of ovalbumin was bound to immunoglobulin inside the oocytes (see Fig. 3*c*).

We have recently demonstrated that MOPC 21 heavy (H) chain trapped in oocytes due to the absence of the complemen-

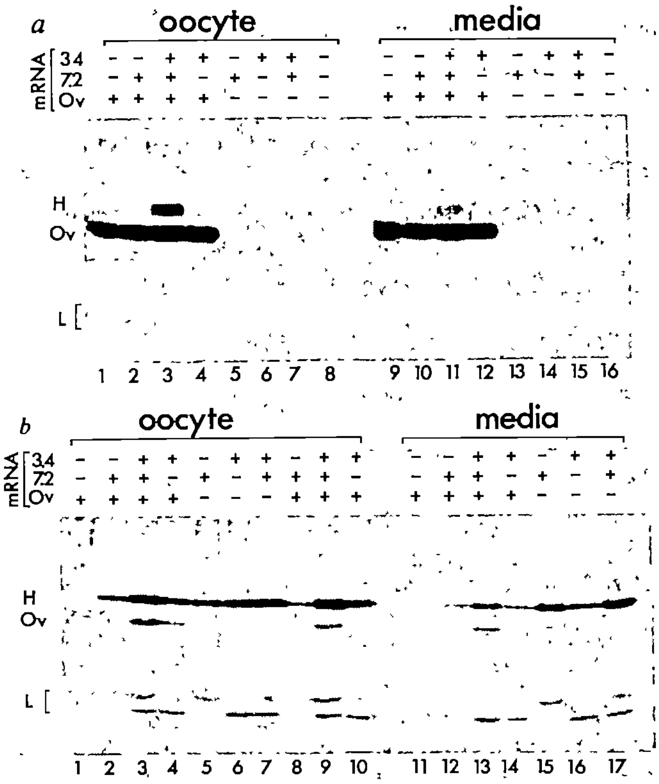

Fig. 2 Coinjection of ovalbumin and anti-ovalbumin mRNAs into oocytes. 7/2 mRNA (0.3 mg ml^{-1}), 3/4 mRNA (0.3 mg ml^{-1}) and ovalbumin mRNA (0 04 mg ml^{-1}) were injected as indicated above into oocytes Oocytes were cultured as described in Fig. 1 and processed for immunoprecipitation with rabbit anti-ovalbumin (*a*) or goat anti-mouse (IgG) (*b*) antibodies In *b* tracks 8–10 homogenization and immunoprecipitation media were supplemented by the addition of unlabelled ovalbumin to 1 mg ml^{-1} final concentration Immunoprecipitates were electrophoresed on 12.5% PAGE gels under reducing conditions and then fluorographed Ov, ovalbumin, H, immunoglobulin heavy chain; L, immunoglobulin light chain.

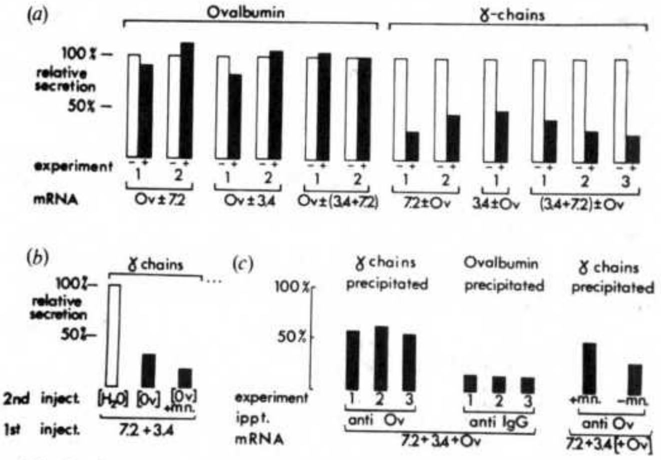

Fig. 3 Quantification of the interactions of monoclonal antibodies with ovalbumin. *a*, Comparisons of the ratios of secreted to intracellular ovalbumin or immunoglobulin γ chains. Appropriate bands from the gels shown in Fig. 2 and other similar experiments were excised and counted in toluene-based scintillant. Corrections were made for radioactivity migrating in the corresponding regions of control tracks. The ratios of secreted to intracellular protein were calculated, converted to percentages and adjusted to 100% for the controls (open blocks) where only one mRNA class (hybridoma or oviduct) was injected. Stippled blocks indicate the injection of both mRNA classes. *b*, Staggered mRNA injection. Appropriate bands from the gel shown in Fig. 4 were treated as above. The mRNAs introduced in the second injection are shown in square brackets. mn, Monensin. *c*, Degree of co-precipitation. The amounts of intracellular ovalbumin or immunoglubulin γ-chain immunoprecipitated with each antibody was calculated by excising gel bands from tracks corresponding to oocyte samples where ovalbumin mRNA, 7/2 mRNA and 3/4 mRNA had been co-injected (for example, Fig. 3, tracks 3; Fig. 5*b*, tracks 12, 13). The amount of the γ chains or ovalbumin which co-precipitated with rabbit anti-ovalbumin or goat anti-mouse IgG respectively is expressed as % of the total amount of each protein directly precipitated by its antibody. The two blocks on the right of this figure correspond to the staggered injection experiment of Fig. 4 with the second mRNA shown in square brackets. mn, Monensin; ippt, immunoprecipitating antibody. When the immunoglobulin tetramers were excised from a non-reducing gel and re-electrophoresed under reducing conditions, band analysis revealed that the heavy chains contained 4–5 times more radioactivity than the light chains. This indicates that the γ-chain contains 4 or 5 methionines or multiples thereof. Using this information and the calculated radioactive contents of γ-chain and ovalbumin (16 methionines) bands in the experiments above, we estimate there to be 1.5 times (or multiples thereof) more ovalbumin than γ-chain in the oocytes.

tary light (L) chain can be rescued by assembly into an H_2L_2 tetramer as a result of an injection of light chain mRNA 24 h later[17]. We were therefore interested to see whether any of the radioactive immunoglobulin molecules remaining in injected oocytes 12 h after the imposition of unlabelled methionine 'chase' conditions were accessible to ovalbumin molecules translated from a post-chase injection of oviduct mRNA. Figure 4 illustrates such a staggered injection experiment. Quantitation of the results shown in Fig. 4 demonstrates first that immunoglobulin secretion was reduced by 60% in the presence of ovalbumin (Fig. 3*b*) and second that when homogenates of such double-injected oocytes were precipitated with rabbit anti-ovalbumin, 29% of the intracellular γ chain co-precipitated, a somewhat lower proportion than the 60% found when the two mRNAs were co-injected (Fig. 3*c*). This value increased to 49% if secretion was further inhibited (Fig. 4, tracks 5 and 6) by use of the ionophore monensin, an agent known to inhibit secretion[18,19] by disrupting the Golgi apparatus[20].

The anti-ovalbumin monoclonal antibodies synthesized by oocytes are clearly specific for ovalbumin after their individual extraction from oocytes. In contrast, intracellular interaction, as identified by immunoprecipitation, between the monoclonal antibodies and ovalbumin can only be shown when both antibodies are present. This might be a consequence of the low affinity of these particular antibodies for their antigenic determinants. Since the two antibodies recognize different determinants an aggregate between ovalbumin and the antibodies is expected. The formation of an aggregate might consolidate the weak interactions between individual molecules[21]. Under

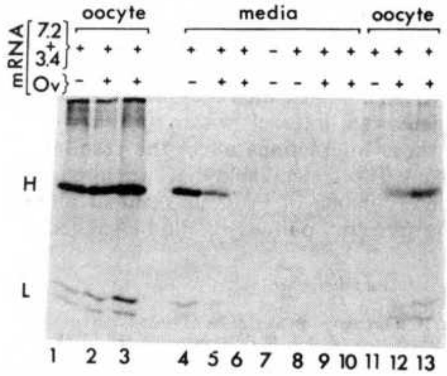

Fig. 4 Staggered injection of ovalbumin and anti-ovalbumin mRNAs. Oocytes were injected with 7/2+3/4 mRNA (both at 0.3 mg ml⁻¹) and immediately cultured overnight in media containing ³⁵S-methionine with (tracks 3, 6, 10, 13) or without 30 μM monensin (Calbiochem). Healthy oocytes were transferred to unlabelled media supplemented with 10 mM methionine for a further 12 h. Ovalbumin mRNA (1 mg ml⁻¹) was then injected into some oocytes and incubation continued for 24 h. Oocytes and media were immunoprecipitated with goat anti-mouse IgG (tracks 1–7) or rabbit anti-ovalbumin (tracks 8–13) antisera and electrophoresed on a 12.5% gel. A 4-day fluorogram is shown. Ov, ovalbumin; H, immunoglobulin heavy chain; L, immunoglobulin light chain.

these circumstances secretion of the tetrameric immunoglobulin was significantly reduced. This is not particularly surprising considering the potentially large size of the aggregate. What is surprising, however, is the reduction of immunoglobulin secretion when only one antibody is present. This reduction is not due to competition with the ovalbumin for some limiting oocyte component necessary for translocation since we have previously demonstrated that mRNA transition itself is the only process which limits the export of secretory proteins from oocytes[11]. Additionally, secretion from oocytes of a murine anti-membrane protein antibody was unaltered by the presence of ovalbumin (Fig. 5). More likely, the reduction of secretion is due to the existence of an equilibrium between slow or unmoving antibody–$(\text{ovalbumin})_2$ trimers and the individual molecules. Even so it is unclear why the movement of such trimers would be reduced unless a conformational change induced by binding in either antigen or antibody is incompatible with the secretory mechanism.

The demonstration that ovalbumin introduced via mRNA injection into oocytes already containing the anti-ovalbumin immunoglobulins can associate with some of the intracellular immunoglobulin indicates that secretory proteins whose

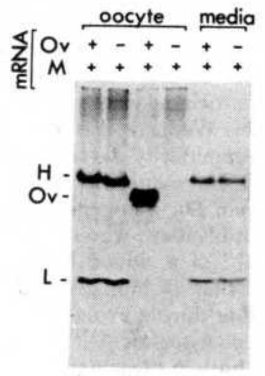

Fig. 5 Secretion of ovalbumin and anti-*Xenopus* immunoglobulins from oocytes. Total RNA was prepared from the murine hybridoma line 18H-11 as described in Fig. 1. This hybridoma secreted an antibody which reacts with a *Xenopus* membrane antigen (E. Jones, unpublished). The RNA at 2 mg ml⁻¹ and ovalbumin mRNA (0.06 mg ml⁻¹) were injected into oocytes and the culture, processing of oocytes and media, and electrophoresis as in Fig. 2. Ov, ovalbumin; M, RNA from 18H-11; H, immunoglobulin heavy chain; L, immunoglobulin light chain; Ippt, immunoprecipitation with rabbit anti-chick ovalbumin (xOv); goat anti-mouse IgG (xIg).

synthesis is temporally separated, can nevertheless become spatially apposed. However, the intracellular location for this interaction remains undetermined.

Thus we have demonstrated that monoclonal antibodies and their antigens can interact within the ER of the oocyte. Evidently these interactions affect the resultant secretion of these proteins. It is conceivable that the function of luminally-exposed determinants of proteins resident along, or passing through the secretory pathway could be gauged by the disruptive effects of introducing monoclonal antibodies raised against them. There seems no reason why this approach cannot be extended to microinjection of cultured cells where the possibilities of combining immunofluorescent and biochemical techniques would facilitate analysis.

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A cDNA sequence encoding a rabbit heavy chain variable region of the V_Ha2 allotype showing homologies with human heavy chain sequences

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There are only four positions in the amino acid sequences of immunoglobulin variable region domains of all species at which the same amino acid is always found[1]. The compiled sequences of rabbit heavy chain variable (V_H) domains have 48 invariant positions[1,2] and at 14 additional positions in the first and third framework segments the different amino acids found correlate with the rabbits' V_Ha allotype[3–5]. This V_H allotypic system with three common 'alleles' *V_Ha1*, *V_Ha2* and *V_Ha3* inherited in simple mendelian fashion[3,4] poses the question of how gene families with distinct sets of conserved codons have evolved and been maintained in the midst of information leading to hypervariability. Here we report a cDNA sequence that encodes a protein characteristic of the rabbit V_Ha2 allotype and we predict the allotype-associated codons that may be found in *V_Ha1* and *V_Ha3* genes. We have also found within the second hypervariable, complementarity-determining region (CDR2) encoded by our cDNA clone, a DNA sequence highly homologous to a human D_H minigene[6]. This observation and a similar one of Wu and Kabat[7], who identified a D_H minigene sequence in the CDR2 of a cloned human V_H gene[8], lends support to their idea that *D* minigene information has contributed to CDR2 either during evolution or ontogeny[7]. Gene conversion[9,10], minigene assortment[11,12] or insertion[13] mechanisms may be involved both in the generation of hypervariability and the conservation of allotype codons.

A partial cDNA library from splenic mRNA of V_Ha2 rabbits infected with *Trypanosoma equiperdum* was the source of clone pμ3. This cDNA clone is a full-length copy of mRNA encoding the hydrophobic leader, V_H and C_H regions[14]. DNA was sequenced by the procedures of Maxam and Gilbert[15]. The variable region restriction map and our sequencing strategy are shown in Fig. 1. Figure 2*a* shows the nucleotide sequence and the deduced amino acid sequence of the variable region of pμ3.

There are no rabbit nucleic acid sequences of cDNAs or genomic V_H, D_H or J_H genes with which to compare our sequence, but we found considerable homology with a cloned human genomic V_H segment, V_H26 (ref. 8) and with the human J_H4 minigene segment[16] and show these for comparison in Fig. 2*a*. Different genomic sequences for human *J* segments encode 15–17 amino acids and through comparison with human J_H4 it is probable that the Phe (TTC) at amino acid position 100C of pμ3 was contributed by a codon from a *J* minigene. The Phe (TTT) preceding it could have been *D*- or *J*-encoded or the site of intracodon recombination between *D* and *J*. The D portion of pμ3 appears to encode seven or eight amino acids (positions 95–100A or 100B) and the sequence encoded bears no resemblance to any published rabbit protein sequences in this region[1,17]. The nucleic acid sequence of our rabbit V_H (Fig. 2*a*) is remarkably homologous to the human V_H26 sequence (73% in the leader and V_H amino acid positions −20 to +94) and to the human J_H4 minigene (80% homology for amino acid positions 100C–113). A striking and unexpected degree of homology is found in the CDR2 of these two sequences. Introduction of a codon gap at amino acid position 52 and deletion of two codons at positions 55 and 56 in the rabbit sequence allows alignment of 17 of 18 bases within this 'hypervariable region'. This is precisely the part of the human CDR2 (amino acid positions 52A–56) in which Wu and Kabat found a sequence of 14 nucleotides homologous to the human *D2* minigene[7]. These 14 nucleotides are enclosed in a box in Fig. 2*a*. As shown in Fig. 2*b*, we find a similar homology although to a lesser degree when the rabbit sequence between the codons for amino acids 49 and 59 is compared with human V_H26 and the human *D1* and *D2* minigene sequences[6]. These observations lead one to ask how it is that a sequence homologous to a *D* minigene is found in the CDR2 of both rabbit and human V_H regions. In mice the hypervariability of the third CDR results from rearrangements that join DNA segments coding for V_H, D_H and J_H during B lymphocyte differentiation[18,19]. The genetic basis of hypervariability of the first and second complementarity-determining regions (CDR1 and CDR2) is less well understood. Wu and Kabat have suggested an insertion mechanism for the generation of hypervariable region diversity[13]. Indeed, the portion of CDR3 encoded by one or more D_H segments of mice is such an insert[18,19]. The present observations lend further but indirect support to the idea that *D* minigene information has somehow contributed to the sequence of the second CDR either through mechanisms of gene conversion[9,10], minigene assortment[11,12] or insertion[13].

The number of J_H genes present in the rabbit genome is unknown. However, published protein sequences that include the segment presumed to be encoded by the J_H segment show

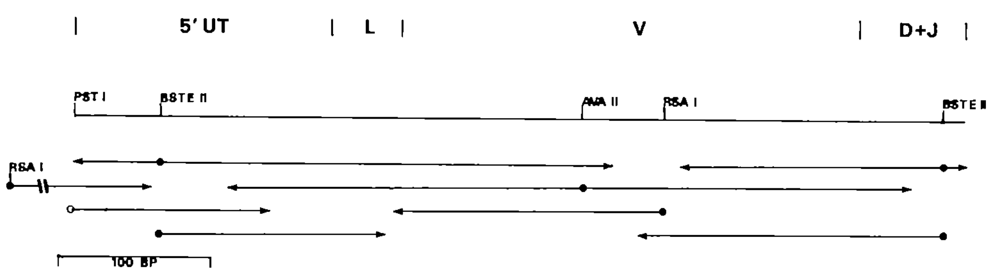


Fig. 1 Strategy used in sequencing the DNA of clone pμ3. The top of the figure indicates the relative sizes of the 5′ untranslated region (5′ UT), the leader sequence (L), the variable region proper (V) and the D and J segments. All sequencing was done using the method of Maxam and Gilbert[15]. Solid and open circles indicate fragments 5′ or 3′ labelled, respectively. The arrows and lines show the length and direction of sequencing. The *Rsa*I site in pBR322 at position 3,847 was also used. The relative size of 100 base pairs (bp) is indicated.

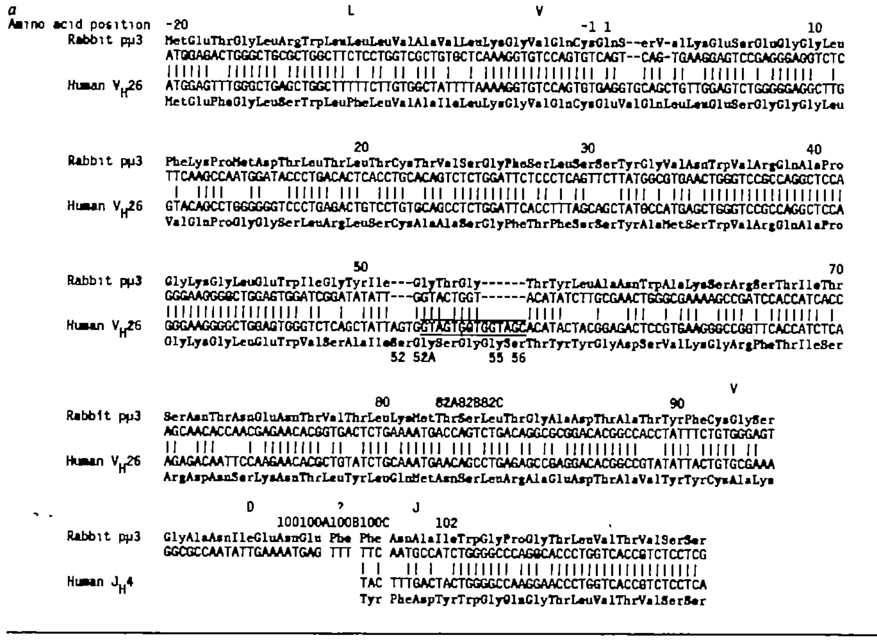
little deviation from a prototype sequence between positions 101 and 113[2,17]. Clone pμ3 encodes the 11 invariant amino acids at positions 103–113 but has Ala replacing Asp and Ile replacing Val at 101 and 102. Whether this is due to a different germ-line *J* segment, somatic mutation or some other mechanism is unknown. The usual Asp and Val can be arrived at by single base changes from the GCC and ATC we observed in clone pμ3.

In our V_H sequence, unusual amino acids are found at positions 93 and 94. This is a highly conserved area of the V_H region and in all other published sequences of rabbit V_H, the amino acids at positions 93 and 94 have been alanine and arginine. Clone pμ3 encodes Gly and Ser at these positions; the sequence was confirmed on both strands. The Gly and Ser codons (GGG, AGT) are potentially derived by single base changes from codons for Ala (GCG) and Arg (CGT). According to Kabat *et al.*[1], of 73 published sequences of V_H regions from all species, 64 have Ala at position 93; Gly has not been reported before. Similarly, 54 of 73 V_H sequences have Arg at position 94 with one reported Ser.

With the exception of the Ala and Arg at 93 and 94 and a deletion at position 2 that is found in 12 of 14 rabbit V_H sequences, each of the 33 other invariant residues listed by Kabat *et al.*[1] are encoded by pμ3. The same pattern is found when considering the amino acids that are thought to comprise the V_Ha allotypes. Although relatively few rabbit V_H proteins have been sequenced, sequence comparisons suggest that the amino acids determining a particular V_Ha allotype are clustered in FR1 and FR3 (positions 5–17 and 65–85). Figure 3*a* summarizes the positions at which V_Ha allotype-associated amino acids occur. Comparison of our deduced amino acid sequence for these positions with known V_H protein sequences clearly shows that clone pμ3 encodes a V_H sequence characteristic of the a2 allotype. As shown in Fig. 3*b*, with two exceptions (a2 Asp to a1 Thr at position 16 and a2 Gly at position 84 to a1 Thr), the codons of pμ3 found at allotype-associated positions can theoretically be changed by one base to give a codon for the amino acids typical of the a1 and a3 allotypes. The codons predicted to occur in a1 and a3 are also compared with those found in the human V_H26 gene (Fig. 3*b*). The codons of V_H26 match the predicted codons at 6 of the 14 positions compared and two additional codons differ only in the third base. Although there have been no confirmed reports of V_Ha-like alleles in man, some rabbit anti-a1 allotype antibodies do react with some human immunoglobulins but do not detect a polymorphic system[20]. The present comparison of the allotypic regions of rabbit with human V_H suggests that 'allelic' variants could occur in man.

It has been suggested that rabbits of different 'allelic' V_Ha phenotypes share the same set of coding sequences but have different allelic regulatory elements[21]. Observations of occasional production of unexpected V_Ha allotypes (for example, V_Ha2 in an a^1a^3 rabbit[22]) have supported such suggestions[22,23]. Our data show that certain portions of the rabbit V_H sequence are highly conserved even between species. Thus a truly allotype-specific V_H probe for investigation of this problem will not be a simple one to design. Clone pμ3 constructed from mRNA of V_Ha2 animals encodes a typical a2 protein sequence and thus provides a means for investigating the genetic basis of the rabbit V_Ha allotypes and solving the paradox of conserved V_H gene families intimately associated with mechanisms that lead to hypervariability.

We thank Myron Waxdal for help with our computer analyses, Dr T. T. Wu who provided computer printouts from his



```
a                                       L                               V
Amino acid position  -20                                                  -1 1                          10
      Rabbit pμ3     MetGluThrGlyLeuArgTrpLeuLeuLeuValAlaValLeuLysGlyValGlnCysGlnS--erV-alLysGluSerGluGlyGlyLeu
                     ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAGT--CAG-TGAAGGAGTCCGAGGGAGGTCTC
                     ||||||  ||||||| |||||||| | || || ||| |  | ||||||||||||||| ||    ||| ||  |||||| | ||||||  |
      Human VH26     ATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTGGCTATTTTAAAAGGTGTCCAGTGTGAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTG
                     MetGluPheGlyLeuSerTrpLeuPheLeuValAlaIleLeuLysGlyValGlnCysGluValGlnLeuLeuGluSerGlyGlyGlyLeu

                                             20                        30                        40
      Rabbit pμ3     PheLysProMetAspThrLeuThrLeuThrCysThrValSerGlyPheSerLeuSerSerTyrGlyValAsnTrpValArgGlnAlaPro
                     TTCAAGCCAATGGATACCCTGACACTCACCTGCACAGTCTCTGGATTCTCCCTCAGTTCTTATGGCGTGAACTGGGTCCGCCAGGCTCCA
                      |  ||||    ||    |||||| |||  ||||  ||| |||||||||| || | ||      |||| | ||| ||||||||||||||||||
      Human VH26     GTACAGCCTGGGGGGTCCCTGAGACTGTCCTGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCA
                     ValGlnProGlyGlySerLeuArgLeuSerCysAlaAlaSerGlyPheThrPheSerSerTyrAlaMetSerTrpValArgGlnAlaPro

                                             50                                                              70
      Rabbit pμ3     GlyLysGlyLeuGluTrpIleGlyTyrIle---GlyThrGly------ThrTyrLeuAlaAsnTrpAlaLysSerArgSerThrIleThr
                     GGGAAGGGGCTGGAGTGGATCGGATATATT---GGTACTGGT------ACATATCTTGCGAACTGGGCGAAAAGCCGATCCACCATCACC
                     ||||||||||||||||| ||  |  ||||    |||| ||||      |||||     |    |||   | |||   |||| | ||||||| |
      Human VH26     GGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGGAGACTCCGTGAAGGGCCGGTTCACCATCTCA
                     GlyLysGlyLeuGluTrpValSerAlaIleSerGlySerGlyGlySerThrTyrTyrGlyAspSerValLysGlyArgPheThrIleSer
                                                   52 52A      55 56

                                                                                          V
                                            80      82A82B82C                     90
      Rabbit pμ3     SerAsnThrAsnGluAsnThrValThrLeuLysMetThrSerLeuThrGlyAlaAspThrAlaThrTyrPheCysGlySer
                     AGCAACACCAACGAGAACACGGTGACTCTGAAAATGACCAGTCTGACAGGCGCGGACACGGCCACCTATTTCTGTGGGAGT
                     ||  |||      | |||||||| ||  |||| |||||| ||| |||| || || ||||||||||    |||| ||||| ||
      Human VH26     AGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAA
                     ArgAspAsnSerLysAsnThrLeuTyrLeuGlnMetAsnSerLeuArgAlaGluAspThrAlaValTyrTyrCysAlaLys

                              D          ?          J
                                 100100A100B100C       102
      Rabbit pμ3     GlyAlaAsnIleGluAsnGlu Phe Phe AsnAlaIleTrpGlyProGlyThrLeuValThrValSerSer
                     GGCGCCAATATTGAAAATGAG TTT TTC AATGCCATCTGGGGCCCAGGCACCCTGGTCACCGTCTCCTCG
                                               | |   || |  |||||||| ||| |||||||||||||||||||
      Human JH4                                TAC TTTGACTACTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCA
                                               Tyr PheAspTyrTrpGlyGlnGlyThrLeuValThrValSerSer
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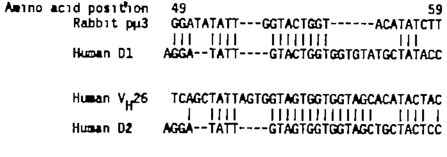

```
b
Amino acid position   49                            59
        Rabbit pμ3    GGATATATT---GGTACTGGT------ACATATCTT
                      |||  ||||    ||||||||         |||
        Human D1     AGGA--TATT----GTACTGGTGGTGTATGCTATACC

        Human VH26    TCAGCTATTAGTGGTAGTGGTGGTAGCACATACTAC
                         |  ||||    |||||||||||||||  |||| |
        Human D2     AGGA--TATT----GTAGTGGTGGTAGCTGCTACTCC
```

Fig. 2 *a*, The nucleic acid sequence and deduced amino acid sequence of the region of clone pμ3 encoding the leader (L), V_H, D_H and *J* segments of a rabbit V_H domain are compared with the human genomic V_H26 (ref. 8) and the human J_H4 (ref. 16) sequences. The amino acid position numbers are those of the standard numbering system of Kabat *et al.*[1]. A sequence of 14 nucleotides in V_H26 that is homologous to 14 bases in the human *D2* minigene is enclosed in a box. The intron between L and V in V_H26 is omitted. Splicing of L to V is believed to occur within Gly −4 (ref. 8). *b*, Comparison of sequences corresponding to amino acid positions 49–59 of rabbit and human V_H sequences in which homologies to human *D* gene sequences are found. The putative *D*-encoded portion of the CDR3 of pμ3 (amino acid positions 95–100A or 100B) is shown in *a*.

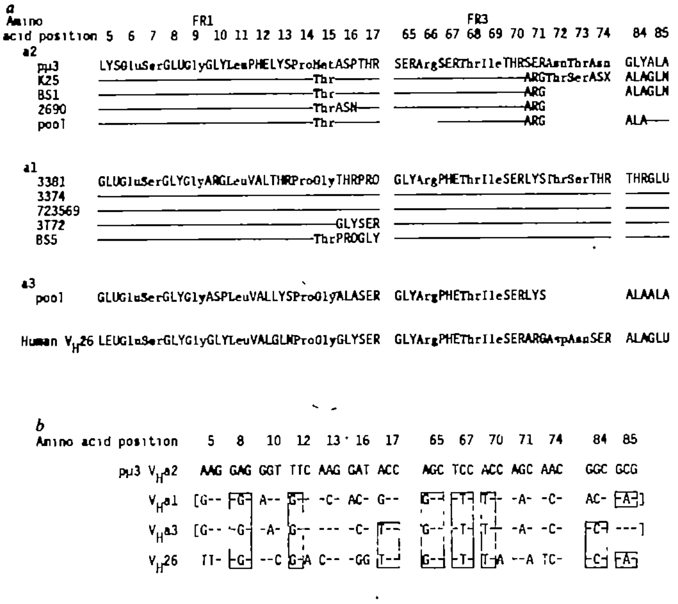
a
Amino acid position 5 6 7 8 9 10 11 12 13 14 15 16 17 65 66 67 68 69 70 71 72 73 74 84 85
FR1
FR3
a2
pμ3 LYSGluSerGLUGlyGLYLeuPHELYSProMetASPTHR SERArgSERThrIleTHRSERAsnThrAsn GLYALA
K25 Thr ARGThrSerASX ALAGLN
BS1 Thr ARG ALAGLN
2690 ThrASN ARG
pool Thr ARG ALA
a1
3381 GLUGluSerGLYGlyARGLeuVALTHRProGlyTHRPRO GLYArgPHEThrIleSERLYSThrSerTHR THRGLU
3374
723569
3T72 GLYSER
BS5 ThrPROGLY
a3
pool GLUGluSerGLYGlyASPLeuVALLYSProGlyALASER GLYArgPHEThrIleSERLYS ALAALA
Human VH26 LEUGluSerGLYGlyGLYLeuVALGLNProGlyGLYSER GLYArgPHEThrIleSERARGAspAsnSER ALAGLU
b
Amino acid position 5 8 10 12 13 16 17 65 67 70 71 74 84 85
pμ3 VHa2 AAG GAG GGT TTC AAG GAT ACC AGC TCC ACC AGC AAC GGC GCG
VHa1 [G-- -G- A-- G-- -C- AC- G-- G-- -T- T-- -A- -C- AC- -A-]
VHa3 [G-- -G- -A- G-- --- -C- T-- G-- -T- T-- -A- -C- -C- ---]
VH26 TT- -G- --C G-A C-- -GG T-- G-- -T- T-A --A TC- -C- -A-

Fig. 3 *a*, Sequences of rabbit V_H proteins of known V_Ha allotype are compared with the deduced amino acid sequence encoded by clone pμ3 in the parts of the first and third framework segments (FR1 and FR3) in which allotype-associated amino acids are found. The sequences are from ref. 1, in which references to the original papers are also given. The amino acids at positions implicated as V_Ha allotype correlates are shown in capital letters. The amino acid sequence encoded by the human *V_H26* gene is also shown for comparison. Solid lines indicate identity to the a2 or a1 prototype sequences. *b*, The codons at amino acid positions implicated as V_Ha allotype correlates found in clone pμ3 (V_Ha2) are compared with hypothetical codons (enclosed between brackets) that would generate amino acids typically found in a1 and a3 proteins. Also compared are the homologous codons found in the human *V_H26* gene sequence. Dashes indicate bases that are identical to those shown for pμ3. Boxes enclose codons in the human *V_H26* sequence and hypothetical a1 and a3 sequences that are identical at all or the first two bases. A one-base change to AGC would also generate a Ser codon at position 17 of *V_Ha3* sequences.

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Domain structure in high molecular weight high mobility group nonhistone chromatin proteins

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The high mobility group (HMG) proteins[1] are a small set of nonhistone chromatin proteins which exhibit preferential affinity for single-stranded DNA[2–4]. Large portions of the amino acid sequences of calf thymus HMG-1 and HMG-2 have recently been published[5]. We have analysed that information and describe here an internal sequence homology in the proteins which, coupled with a prediction of secondary structure, strongly suggests that all the high molecular weight HMG proteins (HMG-1, HMG-2 and HMG-E) exist in three domains of comparable lengths. The N-terminal and central domains have homologous sequences and are probably similar in conformation. The C-terminal domain is nonhomologous and highly acidic. We also present results of trypsin digestion experiments which are consistent with the postulated domain structure. We propose that the N-terminal and central domains of the proteins are DNA-binding regions and that the highly acidic C-terminal domain interacts with histones.

In our first analysis, we have used amino acid sequence comparison methods developed in one of our laboratories[6], which we have previously used on histones[7]. The methods are an adaptation of procedures described by Sankoff[8] for comparison of nucleic acid sequences. The Sankoff algorithm finds optimal alignments of two sequences (or families of sequences in our case) under a specified constraint on the maximum number of gaps. The statistical significance of an alignment proposed on the basis of the Sankoff algorithm is assessed by comparing the scores of the proposed alignment with the scores of alignments between pairs of randomized sequences[7,9]. We have used the scheme of McLachlan for scoring similarities between amino acid residues[10].

HMG-1 and HMG-2 have sufficiently similar amino acid sequences that an alignment between them, as previously published by Walker *et al.*[5], is unambiguous. We therefore treat them as a two-member family[10,11] of homologous sequences, designated HMG-1,2. We have found an internal homology in HMG-1,2 by examining the first two 100-residue portions of the family's sequence. Figure 1 shows an alignment between positions 1–92 and 98–176. The one gap is needed to optimize the alignment before and after a stretch of unknown sequence (positions 40–54). The alignment score in Fig. 1 is 1,079 in the McLachlan scoring scheme as we use it[6]. To assess the statistical significance of the alignment, the Sankoff algorithm was applied to 100 pairs of randomized sequences identical in length and amino acid composition to the sequences of positions 1–97 and 98–189. The scores of the optimized alignments (with one gap allowed) between the pairs of randomized sequences had a mean of 604 and a standard deviation of 57; the highest score observed was 723. Performing more randomizations seemed superfluous, given the large separation between the score of the proposed alignment and the scores of the alignments of randomized sequences. With the data from 100 randomizations, we are limited to concluding[12] that the level of significance of the alignment in Fig. 1 is less than 0.01. The level of significance is, in fact, much lower than 0.01. Although the distributions of scores from comparisons of randomized amino acid sequences are in general not gaussian, for relatively long sequences such as we consider here, the distribution of scores is reasonably close to gaussian[6]. Assuming a gaussian distribution, the proba-

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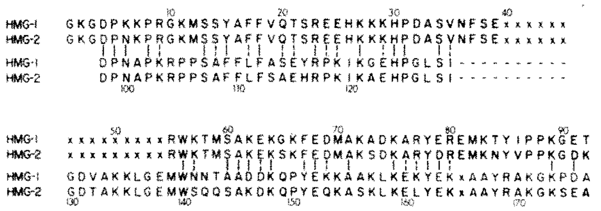
HMG-1 GKGDPKKPRGKMSSYAFFVQTSREEHKKKHPDASVNFSExxxxxx
HMG-2 GKGDPNKPRGKMSSYAFFVQTSREEHKKKHPDASVNFSExxxxxx
HMG-1 DPNAPKRPPSAFFLFASEYRPKIKGEHPGLSI----------
HMG-2 DPNAPKRPPSAFFLFSAEHRPKIKAEHPGLSI----------
10
20
30
40
100
110
120
HMG-1 xxxxxxxxxRWKTMSAKEKGKFEDMAKADKARYEREMKTYIPPKGET
HMG-2 xxxxxxxxxRWKTMSAKEKSKFEDMAKSDKARYDREMKNYVPPKGDK
HMG-1 GDVAKKLGEMWNNTAADDKQPYEKKAAKLKEKYEKxAAYRAKGKPDA
HMG-2 GDTAKKLGEMWSQQSAKDKQPYEQKASKLKELYEKxAAYRAKGKSEA
50
60
70
80
90
130
140
150
160
170

Fig. 1 Internal homology in HMG-1,2. The amino acid sequences of HMG-1 and HMG-2 were treated as a two-member family and numbered as in ref. 5. The sequence at positions 40–53 and at position 165 is unknown (×). The single gap in the alignment is indicated by a series of dashes. The Sankoff algorithm was applied to the first two 100-residue portions of the family's sequence to produce the alignment shown. The 25 positions in the alignment at which the McLachlan score was from 7 to 10 (that is, positions where strong similarity exists between amino acid residues) are indicated by solid lines. The 15 positions at which weaker similarity exists (McLachlan scores of 5 or 6) are indicated by broken lines. Of the remaining 30 aligned positions, only 8 had McLachlan scores less than the average score of 4 obtained by pairwise comparison of all positions in the first homology region with all positions in the second homology region. There are apparently more residues in the stretch of unknown sequence (positions 39–54) in the first homology region than in the corresponding portion of the second homology region (positions 130–139). Thus, when the entire sequence of HMG-1,2 is determined, a gap will be necessary in the second homology region to optimize the entire alignment. In the meantime, we have arbitrarily placed positions 130–139. When allowed two gaps, the Sankoff algorithm introduces a five residue gap in the second homology region after position 164, but without a statistically significant increment[6] in score. We therefore present the one gap alignment.

Table 1 Amino acid composition of fragment P3

	P3 (residues per 10,100 daltons)	HMG-1 (residues in 1–92)
Asp	7.9	5
Thr	2.4	4
Ser	6.1	9
Glu	8.7	12
Pro	4.8	5
Gly	6.2	6
Ala	7.8	7
Cys/2	1.9	1
Val	3.4	2
Met	4.2	4
Ile	0.5	1
Leu	0.6	0
Tyr	3.4	4
Phe	4.8	5
Trp	N.D.	1
His	2.3	2
Lys	17.5	19
Arg	5.8	5

The analysis of P3 was performed after 22 h of hydrolysis at 110 °C in 6 N HCl. No corrections have been made for partial destruction of Thr, Ser, Cys or Tyr. Trp was not determined. The amino acid composition of the N-terminal 92 residues of HMG-1 was calculated from the amino acid sequence of HMG-1 (ref. 5).

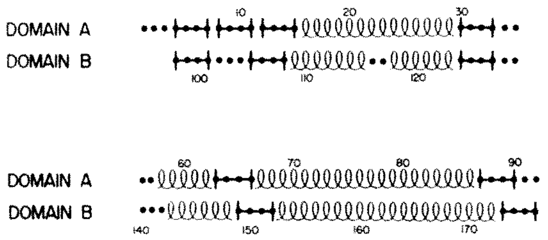

Fig. 2 Predicted secondary structure in domains A and B of HMG-1. The two portions of HMG-1 are aligned as in Fig. 1. Secondary structure, predicted by the rules of Chou and Fasman[17], is shown only for the positions that are paired in Fig. 1. Residues predicted to occur in α-helices are shown as ℓ. •, Nonhelical residues. Sets of four residues predicted to occur as β-turns (probability $p_t \geq 1.2 \times 10^{-4}$) are shown as solid lines. β-sheet potential exists in several short stretches in the HMG-1 sequence, but is not strong enough or does not extend long enough to over-rule α-helical potential in the same regions. The similarities in the predicted secondary structures of domains A and B extend, in the following ways, beyond those that are obvious from the diagram. (1) A third β-turn is predicted in the initial portion of domain B, but is not shown because it has a probability, $p_t = 1.1 \times 10^{-4}$, somewhat lower than the cut-off probability we used for predicting β-turns. In addition, that predicted turn overlaps at residue 101 with the turn predicted in domain B at residues 98–101. (2) The first predicted helical region in domain B is interrupted at positions 116 and 117. A corresponding weakness in the predicted helix occurs in domain A at residues 21–23: the helical potential of those residues is just above the threshold for helix termination. In these positions in each domain, there is β-turn potential, but with probabilities below our cut-off. (3) Residues 36–39 in domain A and residues 130–139 in domain B are not shown in the diagram because they are not aligned in Fig. 1; each region is, however, predicted to be helical. (4) A β-turn is predicted at positions 170–173 in domain B at a probability of 1.25×10^{-4}. The turn, which would correspond almost precisely with a turn predicted in domain A, is not shown because it overlaps with the turn predicted at positions 173–176, which has a higher probability. A similar case holds at positions 146–149 in domain B. Finally, note that positions 55–57 are predicted to be nonhelical because the residues there (Arg, Trp and Lys) all occur with high frequency in nonhelical regions adjacent to N-terminal portions of helices[17]. The final prediction at those positions could be affected by the identity of residues, now unknown, at positions 39–54 in the sequence of HMG-1.

bility of obtaining a score by chance that is 8.3 or more standard deviations from the mean [(1,079–604)/57 = 8.3] is 10^{-16} (obtained by interpolation of data compiled in ref. 13). This very low apparent probability emphasizes that the alignment in Fig. 1 is statistically highly significant.

Much of the sequence of the final portion (positions 177–259) of HMG-1,2 is not yet determined. However, that which has been sequenced is extraordinarily acidic. In HMG-1, over 50% of the positions sequenced are either Glu or Asp[5], and a continuous stretch of 41 acidic residues has been reported[14]. Clearly, the final third of HMG-1,2 is not homologous in amino acid sequence with the two regions shown in Fig. 1.

The above analysis suggests a domain structure for HMG-1 and HMG-2 and, presumably, for the homologous[15] HMG-E. We propose that all the high molecular weight HMG proteins consist of three domains. In HMG-1,2, the first domain, domain A, extends from position 1 to position 92. Domain B, which is homologous in sequence to domain A, corresponds to positions 98–176. Positions 93–97 (Lys-Lys-Lys-Phe-Lys in HMG-1) connect the first two domains. Domain C is the highly acidic C-terminal third of the proteins (positions 177–259). Domains A and B carry a net positive charge at neutral *p*H. In HMG-1, the number of Lys and Arg residues exceeds the number of Asp and Glu residues by 12 and 6 in domains A and B respectively. Domains A and B therefore seem well suited to be DNA-binding domains. Because they contain a normal complement of hydrophobic residues, these domains appear likely to occur in compact, globular conformations. Domain C, with its extreme content of acidic residues and paucity of hydrophobic residues (only six with marked hydrophobic character), presumably occurs as a coil, without organized folding. One would not expect it to bind to DNA and that expectation is supported experimentally[16]. Domain C seems to be designed, instead, to interact electrostatically with positively charged molecules such as histones. It is noteworthy that polyglutamic acid is capable of facilitating the assembly of core histones and DNA into nucleosomes[17].

By applying the Chou and Fasman prediction rules[18] to the sequences of domains A and B, we can predict their secondary structures. The predictions are strikingly similar for the two putative domains (Fig. 2). Each is predicted to have a substantial content of α-helix, in corresponding positions, and no β-sheet. The prediction is therefore consistent qualitatively with estimates of secondary structure in HMG-1 from circular dichroism measurements[16,19]. The similarities in predicted structure extend to the positions of β-turns, even though the residues involved in corresponding predicted β-turns are not generally conserved in sequence between the two homologous regions.

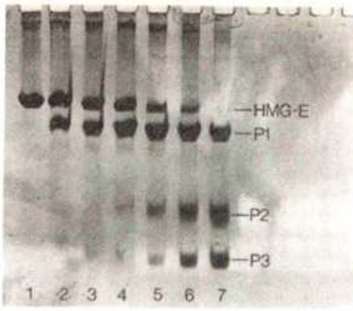

Fig. 3 Acid/urea polyacrylamide gel electrophoresis of the products of trypsin digestion of chicken erythrocyte HMG-E. HMG-E was purified by phosphocellulose chromatography[21] of a 0.35 M NaCl extract of chromatin. The digestion was carried out at 0 °C in 0.1 M NaCl, 10 mM Tris-Cl, *p*H 7.5 with HMG-E at 25 $\mu g\ ml^{-1}$ and trypsin at 0.25 $\mu g\ ml^{-1}$. At the following times (min) aliquots were removed from the reaction mixture and samples prepared for electrophoresis as previously described[4]: 0 (track 1), 2 (track 2), 5 (track 3), 15 (track 4), 30 (track 5), 60 (track 6) and 120 (track 7). Material cleaved from HMG-E in forming P1 has not been detected on either acid/urea or SDS-polyacrylamide gel electrophoresis, perhaps because of failure to react with Coomassie blue or because of rapid diffusion from gels during staining or destaining.

Apart from X-ray crystallographic structure determination, the most direct experimental evidence for domain structure in proteins is the production, by limited proteolysis, of large, discrete fragments that are soluble in aqueous solvents. We have observed such fragments by treating individual high molecular weight HMG proteins with trypsin. Figure 3 shows an electrophoretic analysis of products of the trypsin cleavage of chicken erythrocyte HMG-E. Trypsin digestion mixtures have been subjected to chromatography on phosphocellulose and DNA–cellulose. P1, P2 and P3 are all water-soluble, DNA-binding proteins. We have purified P1 and P3 for analyses. P1 has a molecular weight of 19,000, as determined by sedimentation equilibrium in the analytical centrifuge. The molecular weight of P3 has been estimated to be 10,500 (by SDS-gel analyses) and 10,100 (by sedimentation equilibrium). P1 and P3 both have the same N-terminal amino acid sequence as the parent protein, HMG-E[20]. (Ten residues were identified in HMG-E, in P1 and in P3 by automated Edman degradation.) P1 thus corresponds to domains A and B linked together, and P3 to domain A alone (Table 1). It has not yet proved possible to obtain P2 in sufficient purity or yield for analysis. Because P2 appears in comparable amounts to P3 and with the same time course as P3, and because P2 has a molecular weight of about 10,000 (by SDS-gel analysis), P2 probably corresponds to domain B.

The trypsin digestion results are thus consistent with our postulated three-domain structure for the high molecular weight HMG proteins. Studies of the structure and DNA-binding properties of the trypsin-generated fragments should provide insights into the role of the intact proteins in chromatin structure and function.

We thank Dr George Long for supplying a program for the Chou and Fasman prediction rules, and M. Daniel Land, David Manning, Lisa Wen and William Debold for assistance in some of the experiments. This work was supported by NIH grant R01-GM-13401 to D.C.T., by the Kansas Agricultural Experiment Station and by NIH grants R01–GM–29203, R01-CA-17782 and K04-CA-00425 to G.R.R. Publication 82–265-j, Kansas Agricultural Experiment Station.

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Electron microscopic localization of the replication origin of *Euglena gracilis* chloroplast DNA

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Chloroplast DNA (ctDNA) generally occurs as circular molecules with molecular weights (MWs) in the range $70-130\times10^6$ depending on the species[1,2]. In *Euglena gracilis*, ctDNA (44 µm, 92×10^6 MW[3]) replicates through Cairns-type intermediates[4] having structural aspects suggesting bidirectional replication[5]. Pea and corn ctDNA were shown to contain two displacement loops (D-loops) located 7,100 base pairs (bp) apart[6]. The displacing strands of the two D-loops are located on opposite strands of the parental DNA; they expand towards each other and form a Cairns replicative intermediate when the two strands elongate past each other. Rolling circle intermediates[7], apparently resulting from the continuation of Cairns rounds of replication, have also been observed[8]. The origins of replication were not located on physical maps for any of these studies. We present here the results of an electron microscopic (EM) study indicating that replication of ctDNA in *Euglena* is initiated near the 5′ end of the supplementary 16S ribosomal RNA gene[9].

Replicating ctDNA was extracted from *Euglena* cells synchronized by light–dark cycles (12 h/12 h)[5] (Fig. 1). The highest yields of replicating molecules (up to 20% of the circular molecules) were obtained from cells collected in the middle of the light phase (compare with results of refs 5, 10). The replicated fraction of the various molecules represented from 1 to 80% of the total genome length.

When replicating ctDNA was digested with the restriction endonuclease *Pvu*II, replication loops were observed on fragments 22.4 ± 0.3 µm long ($n=31$ molecules) (Fig. 1*a*, *b*) corresponding to the fragment *Pvu* A (67 kilobase pairs, kbp; Fig. 2). After *Xho*I digestion replication loops were localized on fragments 16.2 ± 0.3 µm long ($n=14$) (Fig. 1*c*) corresponding to the fragment *Xho* A (49 kbp; Fig. 2). These observations, taken together show that replication is initiated in the region common to *Pvu* A and *Xho* A fragments, that is, in the region 5′ to the ribosomal cistrons (see physical map; Fig. 2). To ascertain this localization, R-loops were formed by hybridizing 23S ribosomal RNA (rRNA) with *Pvu*II digests of replicating ctDNA. In agreement with the restriction map of Fig. 2, R-loops were observed on *Pvu* A fragments only. The region containing the three 23S cistrons is 4.7 ± 0.1 µm long ($n=14$) while the adjacent segments spanning 3′ and 5′ of

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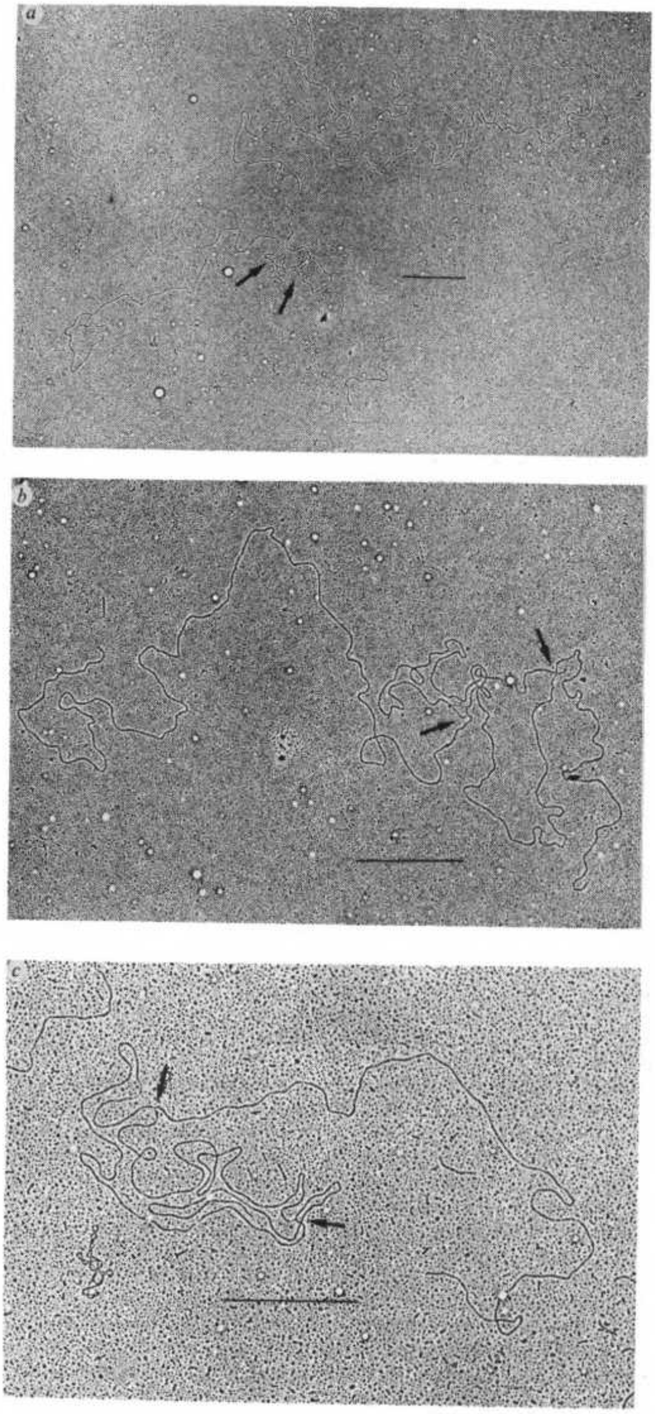

Fig. 1 Restriction fragments bearing replication loops. Synchronous cultures of *Euglena gracilis* Z (strain 1224–5/25 from Göttingen Algensammlung) were grown phototrophically on mineral medium[29] plus vitamins B_1 and B_{12} and aerated with air/CO_2 (95:5) in 20-l carboys at 27 °C. Cultures were synchronized by light–dark cycles (12 h/12 h; 5,000 lux) and were collected between 6 and 8 h after onset of the light phase at densities of $2\text{–}2.5 \times 10^5$ cells ml^{-1}. Chloroplasts were isolated by sucrose flotation according to ref. 3. Replicating DNA was isolated by CsCl/ethidium bromide gradient centrifugation[5]. After digestion with *Pvu*II (Boehringer) or *Xho*I (BRL), DNA was mounted for electron microscopy by the formamide technique[30], with pBR322 plasmid as internal standard. Grids were stained with uranyl acetate and rotary-shadowed with Pt/Pd (80:20). Calibrations of the electron microscope were made using a diffraction grating replica (2,160 lines mm^{-1}; Agar Aids). Arrows indicate the replication forks on fragment of ctDNA digested by *Pvu*II (*a*, *b*) or *Xho*I (*c*). The extent of replication is *a*, 3%; *b*, 13.5%; and *c*, 12.5% of the entire ctDNA molecule. In *a* and *b* one of the Cairns forks shows branch migration[31]. Scale bar, 1 μm.

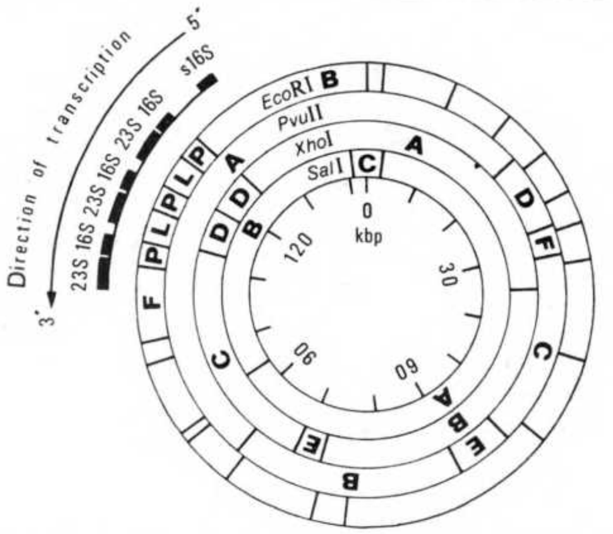

Fig. 2 Physical restriction map of *Euglena* ctDNA from Hallick[12]. s16S is the supplementary 16S rRNA gene[9].

these ribosomal genes are, respectively, 6.2 μm and 11.5 μm long. When *Pvu* A fragments simultaneously carrying R-loops and a replication loop were observed, the replication loops were found on the 11.5 μm-long side of the *Pvu* A fragments (13 molecules observed; Figs 3, 4). These results demonstrate that replication is initiated in a unique region of the ctDNA molecule 5′ to the ribosomal genes.

The two branches of the replication loops appear to be double-stranded except for a region of variable length on one of the branches at each of the replication forks, as already observed by Richards and Manning[5]. When replication covers the ribosomal region totally or partially, both branches of the replication bubble carry R-loops (Fig. 3*b*, *c*). This demonstrates that both ctDNA strands are replicated simultaneously in *Euglena*. In contrast, a large fraction of the H-strand of mitochondrial DNA is replicated unidirectionally before replication of the L-strand starts[11].

In Fig. 4, we have aligned 35 molecules bearing replication loops. The top of the histogram, assembled from the replication loops of *Pvu* A fragments (locus 0 in Fig. 4*a*) is located 11 kbp from the 5′ end of the first 23S cistron (taking 14 kbp for the length of the 23S region[12]), that is, about 5 kbp from the 5′ end of the supplementary 16S gene. The shape of the histogram is fairly symmetrical, suggesting that the replication is essentially bidirectional; however, the origin of replication thus determined is not necessarily located at the centre of the replication loops observed on individual molecules. One must assume that the migration rates of the two Cairns forks may differ widely from each other in some molecules, yielding almost unidirectional replication. In some molecules observed, the origin of replication determined by the histogram is not located in the replication loop. This cannot be due to experimental errors of measurement (see Fig. 4*b* for reproducibility of 23S cistrons positioning). Our results, however, do not exclude the possibility of two neighbouring replication origins as described for pea and maize[6]. Moreover, replication is initiated in a region demonstrated to be heterogeneous[13] (region *Bgl* Z) and to contain an inverted repeat of variable length[14]. Thus, it is possible that the variability observed in replication figures may result from the structural variability of this region.

Note for comparison that the replication origin is close to the ribosomal cistrons in various mitochondrial genomes (rat[15], mouse[16], man[17], *Xenopus*[18] and *Drosophila*[19]), and is associated with inverted repeated structures in several replicons (mitochondria[11], *Escherichia coli*[20], lambdoid coliphages[21], simian virus 40[22] and the 2 μm plasmid of yeast[23]). The mitochondrial genome of wild-type yeast cells apparently contains several putative origins of replication, one of them at least

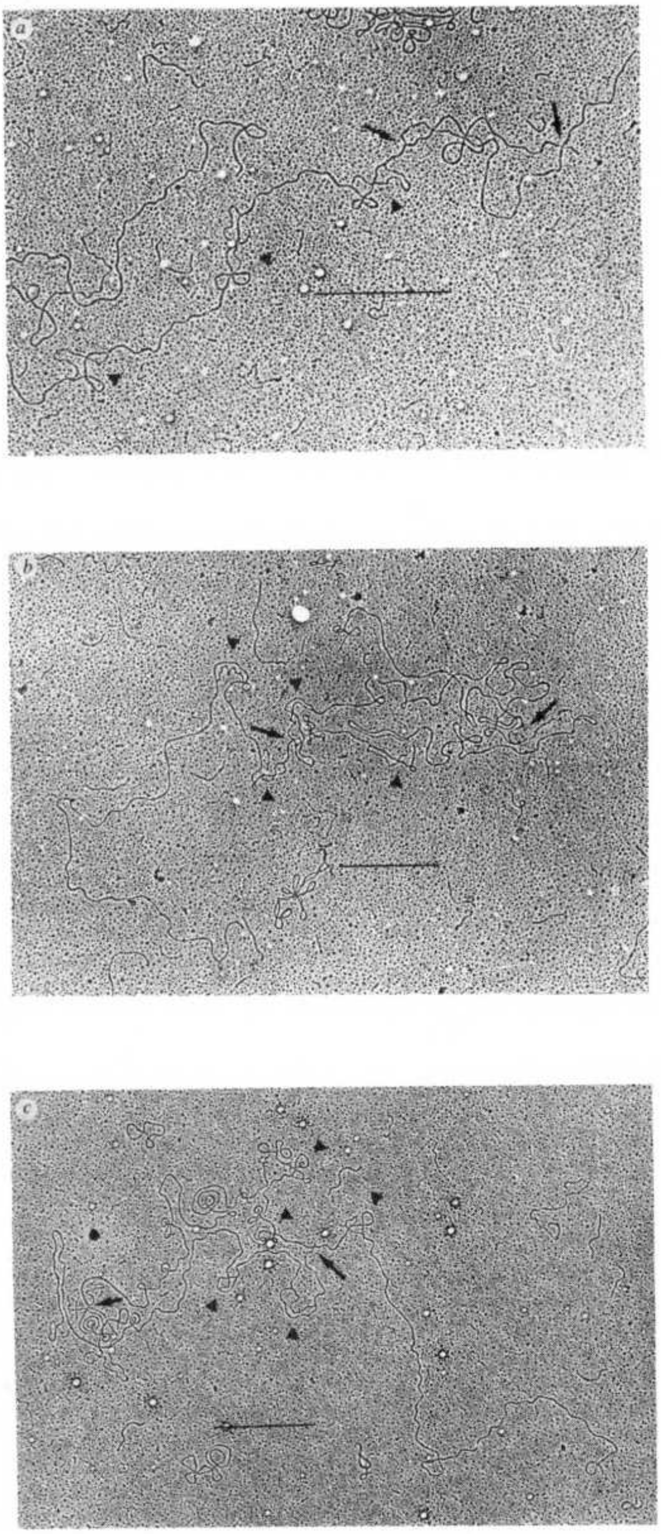

Fig. 3 *Pvu* A restriction fragments bearing simultaneously R-loops and a replication loop. ctDNA was prepared, digested and spread as described for Fig. 1. rRNA was phenol-extracted from SDS-lysed chloroplasts and 23S RNA was purified by 1.5% agarose gel electrophoresis, electroelution and alcohol precipitation. R-loops were formed according to Wellauer and Dawid[23] (incubation for 16 h at 45 °C). Arrows indicate R-loops. *a*, Replication concerns 5% of the total genome but not the ribosomal cistrons. A details of the ribosomal and replicated region is shown. *b*, Replication concerns 18% of the genome; the first 23S cistron has been replicated. *c*, 29% of the total genome and two 23S cistrons have been replicated. These molecules show branch migrations at one replication fork. Small circular molecules are pBR322 plasmid DNA. Scale bar, 1 μm.

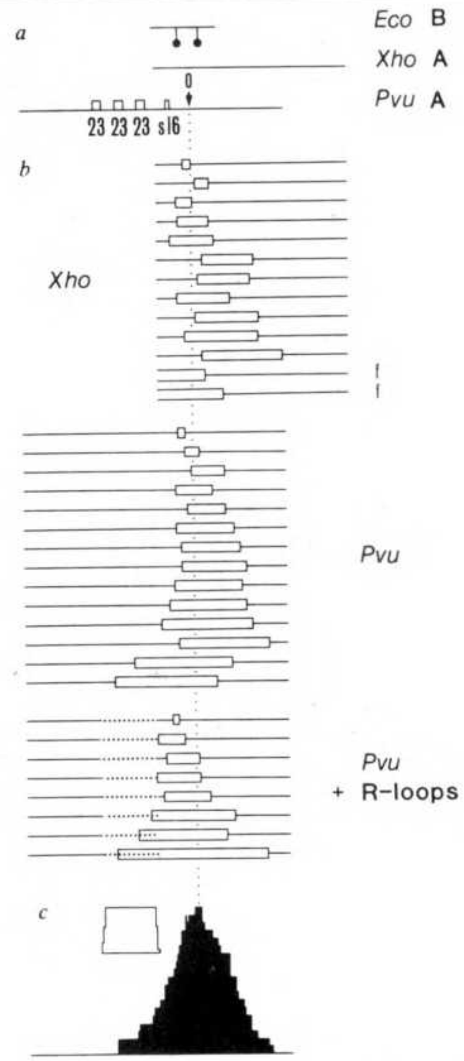

Fig. 4 Alignment of replication patterns with the physical restriction map. *a*, Relative positions of restriction fragments *Pvu* A, *Xho* A and *Eco* B from the physical map of Hallick[12] (Fig. 2). ● Represents the limits of the *Bgl* Z region as defined by Jenni *et al.*[13]. The positions of ribosomal 23S and supplementary 16S cistrons are indicated on the *Pvu* A fragment; 0 corresponds to the top of the histogram from *c*. *b*, Restriction fragments carrying replication loops. Restriction fragments were analysed by measuring the non-replicated portions and both branches of the replication loops of each individual restriction fragment. For all molecules observed, the two branches of the replication loops were identical in length (± 5%); individual restriction fragments had total lengths within ±7% of the average fragment length. Only replication loops larger than 0.5 μm were taken into account to avoid possible confusion with denaturation loops, which are generally rare and smaller than 400–500 bp (~0.15 μm). Open boxes represent the replication bubbles; dotted lines represent the region covered by 23S R-loops. In each group molecules are arranged according to increasing extent of replication. f, Forked molecules. *c*, Histogram of cumulated *Pvu* A fragments from *b*. The open histogram indicates the position of the 23S ribosomal region observed on *Pvu* fragments.

being carried by each of the segments excized and repeated in spontaneous petites mutants[24]. In an analogous way, ribosomal DNA amplification in *Tetrahymena* seems to proceed after excision of a rDNA segment from the micronucleus chromosomal DNA during conjugation, followed by extensive replication of extrachromosomal rDNA molecules due to the presence of an origin of replication located on the excized segments[25]. Similarly, the tandem arrangement of the ribosomal cistrons in *Euglena*, together with their close proximity to the replication origin, could explain the relative amplification of these ribosomal cistrons described during mutagenesis[26] or suggested during the cell cycle[27,28].

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Impaired nuclear transport of a human variant tRNA$_i^{Met}$

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Recently, a human variant tRNA$_i^{Met}$ (initiator tRNA^{Met}) gene has been cloned from a recombinant library of fetal liver DNA[1]. This gene contains a G to T transversion within the highly conserved TCGA sequence in loop 4 ('Tψ loop'), a sequence position occupied exclusively by a purine (usually G) in almost 200 prokaryotic and eukaryotic tRNAs[2]. Recent studies with the cloned gene, both *in vitro*[3] and when expressed in an intact cell on a simian virus 40 viral vector[4], have shown that one functional consequence of this base substitution is a reduction in the rate of processing of the primary transcript of the gene. This reaction involves sequential excision of 5′- and 3′-terminal sequences[3]. Here we show that following microinjection of the variant tRNA$_i^{Met}$ gene into the germinal vesicle of the intact *Xenopus laevis* oocyte, the primary transcript is slowly but accurately processed to a mature variant tRNA$_i^{Met}$ species which remains trapped within the oocyte nucleus, its escape apparently being prevented by the nuclear membrane. This is the first evidence that the transport of a tRNA molecule can be blocked by a point mutation. The results suggest that a selective tRNA nuclear transport mechanism exists in the eukaryotic cell and identify a critical role for the highly conserved Tψ loop in this process.

The *X. laevis* oocyte has been shown to accurately transcribe and process several homologous and heterologous tRNA genes[5–9] and provides a system for studying the intracellular distribution of intermediates in tRNA biosynthesis. After microinjection of either a cloned tRNA gene or a purified transcript, the oocyte can be manually dissected and the RNA species within the nucleus and cytoplasm analysed separately[8,9]. The intracellular distribution of tRNA intermediates is preserved during manual dissection, in contrast to the 'leakiness' of somatic nuclei with respect to these species[10], when isolated by standard aqueous fractionation.

The DNA sequences of the two human tRNA$_i^{Met}$ genes used in this study are co-linear with that of mature tRNA$_i^{Met}$ and neither contains an intervening sequence[1]; gene 1 is a variant differing from the common vertebrate tRNA$_i^{Met}$ sequence by a G to T transversion at position 56 in the DNA sequence (corresponding to position 57 in the standard tRNA numbering scheme[2]), and gene 2 contains the common vertebrate sequence. The 5′ and 3′ flanking sequences of the two genes share only limited homology, with the corresponding primary transcripts of each gene differing in both the sequence and length of the 5′ leader and 3′ trailer[3]. Plasmids phH1E (gene 1) and phH2D (gene 2), described in detail elsewhere[1], each contain a human chromosomal fragment bearing a single tRNA gene.

The two plasmids were microinjected separately into the germinal vesicle of stage 6 *X. laevis* oocytes. Five hours after injection of the gene plus [α-^{32}P]GTP, RNA was extracted and

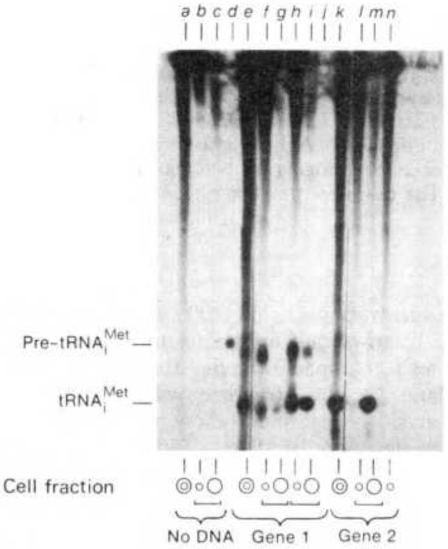

Fig. 1 Intracellular distribution of ^{32}P-labelled RNA species in *X. laevis* oocytes after microinjection of human tRNA$_i^{Met}$ genes. Ovaries were collected from gravid *X. laevis* females and microinjected as described elsewhere[5]; ~20 nl of solution containing 10 ng of plasmid were injected per nucleus. Plasmids phHIE (gene 1) and phH2D (gene 2) have been described in detail elsewhere[1]. Plasmids were isolated by acid–phenol extraction[18] followed by gel filtration over Sepharose 4B. After injection of the plasmid, 50 nl of [α-^{32}P]GTP (10 mCi ml^{-1}; SA 760 Ci mmol^{-1}, NEN) were injected in a separate step into either the vegetal (white) pole or the equator. Oocytes were maintained at 21 °C for 5 h. RNA was extracted from either the whole oocyte or the separated germinal vesicle and cytoplasm, manually dissected as described by Melton *et al.*[9]. RNA was electrophoresed on a 10% acrylamide gel containing 7 M urea and autoradiographed for 12 h as described[3]. No exogenous DNA injected: *a*, whole oocyte; *b*, germinal vesicle; *c*, cytoplasm. phHIE (gene 1) injected: *e*, whole oocyte; *f*, *h*, germinal vesicles; and *g*, *i*, corresponding cytoplasmic fractions. phH2D (gene 2) injected: *k*, whole oocyte; *l*, *n*, germinal vesicle; and *m*, corresponding cytoplasm (cytoplasmic fraction associated with the vesicle in lane *n* is not presented). Lanes *d* and *j*, ^{32}P-labelled primary transcripts of gene 1 and gene 2, respectively, synthesized *in vitro*[3] (see Fig. 3 legend), were included as size markers.

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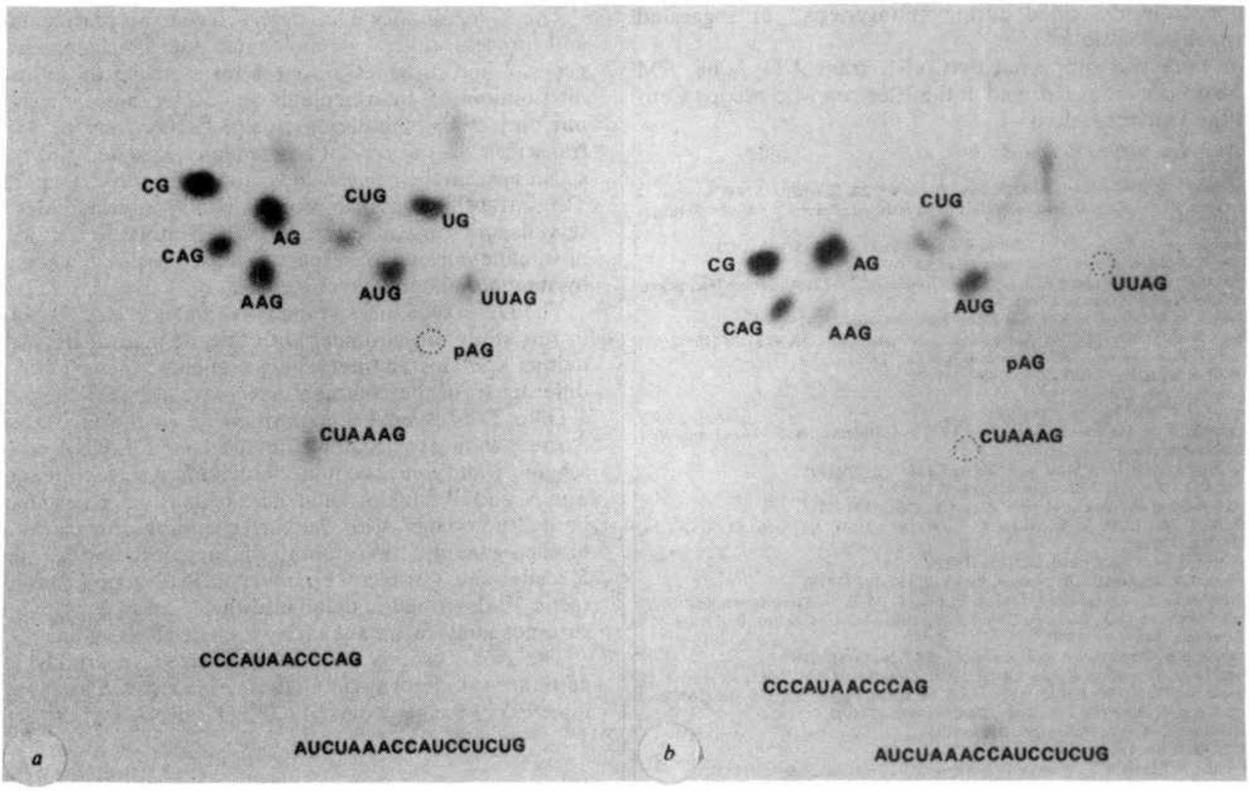

Fig. 2 RNase T_1 oligonucleotide fingerprint of gene 1 pre-$tRNA_i^{Met}$ and intranuclear $tRNA_i^{Met}$. RNA species were extracted from the gel shown in Fig. 1*f* and fingerprinted by classical techniques as described elsewhere[3]. Expected positions of certain oligonucleotides not observed in each fingerprint are indicated by dotted circles. *a*, Pre-$tRNA_i^{Met}$ transcribed from gene 1. The long oligonucleotide characteristic of the variant, AUCUAAACCAUCCUCUG, results from the elimination of an AUCG in loop 4 due to base substitution of G for U. The oligonoucleotide CUAAAG can also be seen, bridging the 3′ end of the mature sequence and 3′ trailer. UUAG arises from position −2 in the DNA sequence, bridging the two nucleotides of the 5′ leader and two nucleotides from the start of the mature sequence. *b*, Intranuclear $tRNA_i^{Met}$. The characteristic long oligonucleotide is present, demonstrating the presence of the mutation within the loop 4 region. The 3′ bridging sequence, CUAAAG, is absent and the mature 5′ end, pAG, is present.

analysed by electrophoresis in 10% acrylamide gels in 7 M urea. Figure 1 shows that injection of gene 2 resulted in the synthesis of an RNA species having the mobility expected for $tRNA_i^{Met}$ (lane *k*); the structure was confirmed by RNA sequence analysis as described below. On injection of gene 1, two major species were produced (Fig. 1*e*); the slower is the precursor of the variant tRNA while the faster, major species, is mature variant $tRNA_i^{Met}$.

To determine the intracellular distribution of the transcripts of the normal and mutant $tRNA_i^{Met}$ genes, the gene plus [α-^{32}P]GTP were injected into the germinal vesicle of an intact oocyte and kept at 21 °C for 5 h; the oocyte was then manually dissected and RNA species extracted from the separated nucleus and cytoplasm. The $tRNA_i^{Met}$ species synthesized from the normal gene was found principally in the cytoplasmic compartment (Fig. 1*m*) with about 1% of the total newly synthesized $tRNA_i^{Met}$ remaining in the germinal vesicle (Fig. 1*l*, *n*). As the volume of the germinal vesicle is about 10% that of the oocyte[11], the intranuclear concentration is lower than would be expected from free diffusion of the species between nucleus and cytoplasm and suggests that the $tRNA_i^{Met}$ partitions preferentially in the cytoplasm.

The intracellular distribution of the RNA species transcribed from the mutant gene differed strikingly from that observed for normal $tRNA_i^{Met}$. As seen in the representative pair of oocyte dissections, the germinal vesicle contained, in addition to the precursor species, between 80% (Fig. 1, lanes *f*, *g*) and 60% (Fig. 1*h*, *i*) of the newly synthesized variant tRNA. Because tearing of the germinal vesicle membrane leads to leakage of the intranuclear tRNA species, as we show below, we cannot exclude the possibility that the cytoplasmic component was solely an artefact of isolation. Note, however, that the relative proportion of the tRNA species present in each compartment did not change significantly with time up to 18 h.

The putative variant tRNA precursor, and the nuclear and cytoplasmic tRNA species were isolated and analysed by classical two-dimensional T_1 RNase oligonucleotide fingerprinting[12]. The fingerprints generated were compared with previous analyses of the *in vitro* transcripts of this gene[3]. The fingerprint of the slowly migrating species (Fig. 2*a*) is characteristic of the precursor, while that of the intranuclear tRNA (Fig. 2*b*) is characteristic of the mature variant $tRNA_i^{Met}$. Fingerprint analysis of the tRNA synthesized in the presence of [α-^{32}P]CTP revealed an oligonucleotide with location and composition consistent with the mature 3′-terminal oligonucleotide $CUACCA^{OH}$, demonstrating terminal CCA addition on the accurately processed terminus (data not shown). A fingerprint of the variant tRNA recovered from the cytoplasm appeared identical to that of the intranuclear species (data not shown).

To determine whether the processing and transport behaviour of the mutant tRNA could be demonstrated with purified RNA species, ^{32}P-labelled primary transcripts of each gene synthesized *in vitro*[3] were injected separately into individual oocytes. After 5 h incubation, the RNA was extracted from either the whole oocyte or from the germinal vesicle and cytoplasm after manual dissection. Injection of the purified primary transcripts resulted in a similar appearance and intracellular distribution of species observed during active transcription in the oocyte. Thus, the primary gene 2 transcript was fully converted to $tRNA_i^{Met}$ (Fig. 3*k*) 5 h after nuclear injection. The processing nucleases appear to be intranuclear, because injection of the same quantity of precursor into the cytoplasm resulted in less efficient conversion (Fig. 3*l*). Analysis of the distribution of the $tRNA_i^{Met}$ species revealed that virtually

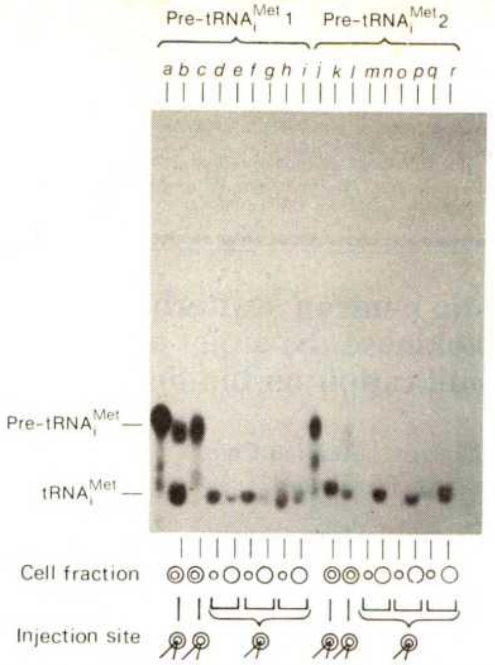

Fig. 3 Intracellular processing and distribution of ^{32}P-labelled tRNA$_i^{Met}$ primary transcripts after microinjection of the purified RNA into *X. laevis* oocytes. The primary transcripts of gene 1 and gene 2 were synthesized *in vitro* using a partially purified KB cell postnuclear fraction as described previously[3], and have been shown to be identical in sequence to the species synthesized in the *X. laevis* oocyte extract[3]. The gene 1 primary transcript, 90 nucleotides long, contains a 3-base 5′ leader and a 13-base 3′ trailer, while the 89-base gene 2 transcript contains an 8-base leader and trailer[3]. The RNA species were purified from the transcription reactions by phenol extraction, concentrated by ethanol precipitation, and then resuspended in a small volume of 88 mM NaCl, 20 mM Tris-HCl *p*H 8.0. RNA solutions were injected into oocyte nuclei, as described in Fig. 1 legend, or into the cytoplasmic pole. Following incubation at 21 °C for 5 h, RNA was extracted from either whole oocytes or nuclear and cytoplasmic fractions, obtained as described in Fig. 1 legend. *a*, Primary transcript of gene 1 synthesized *in vitro*. *b*, Whole oocyte, following injection into the germinal vesicle of the amount of ^{32}P-RNA shown in *a*. *c*, Whole oocyte, following injection into the cytoplasm. *d, f, h*, Germinal vesicles; *e, g, i*, corresponding cytoplasmic fractions following germinal vesicle injections. Lanes *j–r* show corresponding data obtained after injection of the primary transcript of gene 2.

no tRNA$_i^{Met}$ remained within the nucleus; it was localized within the cytoplasm, as shown in three sets of single oocyte dissections (Fig. 3*m*/*n*, *o*/*p* and *q*/*r*).

The primary transcript of gene 1 was processed to a tRNA species more slowly than that of gene 2, and the variant tRNA$_i^{Met}$ was inefficiently transported from the oocyte nucleus. About 50% of the primary transcript of gene 1 was processed to a tRNA by 5 h after microinjection into the germinal vesicle (Fig. 3*b*). We estimate that the rate of processing of this transcript is about 5% of that observed for the gene 2 species, since 50% conversion of the gene 2 transcript occurred within about 15 min after microinjection (data not shown). As demonstrated in three individual oocyte dissections, between 90% (Fig. 3*d*/*e* and *f*/*g*) and 60% (Fig. 3*h*/*i*) of the processed mutant tRNA$_i^{Met}$ remained within the germinal vesicle 5 h after intranuclear injection of the gene 1 transcript. The gene 1 primary transcript was not significantly processed after injection into the cytoplasm of the oocyte (Fig. 3*c*), which demonstrates that the primary transcript of the variant gene cannot efficiently enter the nucleus from the cytoplasmic compartment during this period.

To determine the mechanism by which the tRNA$_i^{Met}$ remains sequestered within the nucleus of the oocyte, we asked whether this mutant tRNA was limited from free diffusion by the nuclear membrane, by physical attachment to a cellular component such as the membrane itself or the intranuclear matrix, or due to its association with a large soluble marcomolecule which might itself be limited from leaving the germinal vesicle. Five hours after injection of gene 1 and [α-^{32}P]GTP, the germinal vesicles were manually isolated and placed in a small volume of J buffer[13] (70 mM NH_4Cl, 7 mM $MgCl_2$, 0.1 mM EDTA, 2.5 mM dithiothreitol, 10 mM HEPES *p*H 7.4, 10% glycerol) or the same buffer containing 1% Triton X-100. [In the presence of this non-ionic detergent the nuclear membrane is disrupted without apparent effect on the basic overall structural integrity of the intranuclear matrix, and the surrounding pore lamina system or 'envelope'[14]. Figure 4 (inset) shows a low power magnification of a germinal vesicle suspended for 30 min at 21 °C in J buffer containing Triton X-100. No appreciable difference in gross morphology was observed between the germinal vesicle thus treated and one suspended in J buffer alone for this period of time.] After incubation of the isolated germinal vesicle in J buffer, the labelled RNA species remained within the nucleus (Fig. 4*c*) with little RNA found in the surrounding buffer (lane *d*). In contrast, treatment of the germinal vesicle with the solution containing Triton X-100 resulted in the almost complete release of the RNA species from the nucleus (Fig. 4*e*) to the surrounding solution (lane *f*).

Thus, treatment of the germinal vesicle in conditions which lead to dissolution of the nuclear membrane without gross physical disruption of the nucleus, results in leakage of the

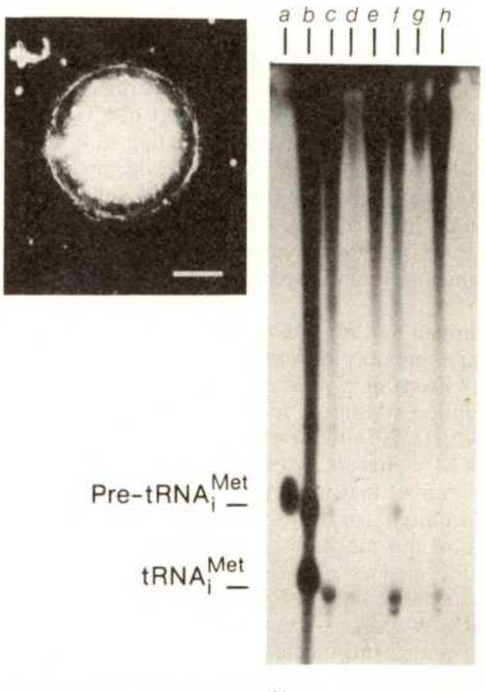

Fig. 4 Intranuclear variant tRNA$_i^{Met}$ is trapped in the germinal vesicle by the nuclear membrane. ^{32}P-RNA was synthesized in oocytes after injection of phHIE (gene 1) as described in Fig. 1 legend. *a*, Gene 1 primary transcript synthesized *in vitro*. *b*, RNA extracted from whole oocyte. Germinal vesicles were isolated as described in Fig. 1 legend and suspended in 300 µl of either J buffer[13] or J buffer containing 1% Triton X-100, and maintained at 21 °C for 30 min, at which time the RNA species present in both nucleus and surrounding buffer were analysed. Germinal vesicle suspended in J buffer alone: *c*, germinal vesicle; *d*, surrounding fluid. Germinal vesicle suspended in J buffer plus Triton X-100: *e*, germinal vesicle; *f*, surrounding fluid. One germinal vesicle was placed in J buffer and the nuclear membrane manually separated from the nucleoplasm: g, RNA associated with nuclear membrane; *h*, intranuclear contents. Inset, *X. laevis* germinal vesicle after incubation at 21 °C for 30 min in J buffer containing 1% Triton X-100. No change in gross morphology of the germinal vesicle was observed after detergent treatment. Note the nuclear envelope, nucleoplasmic gel, and numerous amplified nucleoli. (Nomarski phase optics; scale bar, 0.1 mm.)

variant tRNA$_i^{Met}$. To determine whether the RNA species is released as a result of the membrane acting as a diffusion barrier or as a result of previous attachment of the tRNA species to the membrane, a germinal vesicle, previously injected with gene 1 and [α-^{32}P]GTP, was placed in J buffer (not containing Triton X-100) and manually stripped of its enclosing nuclear envelope as described elsewhere[13]. When the contents of the nucleus were separated from the membrane, the variant tRNA$_i^{Met}$ partitioned with the nucleoplasmic contents (Fig. 4*h*) rather than the membrane (lane *g*).

Velocity sedimentation analysis of the intranuclear contents of the manually stripped germinal vesicle revealed that the intranuclear tRNA$_i^{Met}$ species has a mobility of ~4S (data not shown). This experiment demonstrates that the species is not associated within the nucleus in a large macromolecular complex (such as the 42S particle[15]) and, even in the absence of detergent treatment, is not bound tightly to such components as the nuclear gel, the intranuclear scaffold, or chromatin (nuclear and nucleolar).

We have demonstrated here that a human variant tRNA$_i^{Met}$ containing a base substitution within the highly conserved loop 4 region is transported inefficiently from the oocyte nucleus. Although only tRNA species having mature 5′ and 3′ termini can leave the nucleus, as noted here and in an earlier study[9], our data reveal that accurate nucleolytic processing of a tRNA molecule will not guarantee nuclear escape. In addition, the trapping of the variant tRNA$_i^{Met}$ within the oocyte nucleus demonstrates that the nuclear membrane can restrict the diffusion of a mature tRNA species and provides evidence for the existence of a tRNA transport mechanism. The behaviour of the variant tRNA$_i^{Met}$ strongly suggests that the highly conserved UCGA sequence within the loop 4 region of eukaryotic tRNA has a role in the overall transport of a tRNA molecule across the nuclear membrane. Our finding that the variant is processed slowly in addition to being transported inefficiently suggests that the mechanisms of processing and transport might be functionally inter-related. Alternatively, both processing and transport may operate by entirely independent mechanisms, each recognizing common sequences (or structure) in the tRNA molecule.

Is this human variant tRNA$_i^{Met}$ a 'pseudogene' in the sense of an inactive member of a multigene family, or might it have a function? From *in vitro* studies of several RNA polymerase III transcription systems it appears that feedback inhibition by either tRNA (U. Affolter and M. Z., unpublished observations) or 5S rRNA[16,17] occurs, presumably by the binding of RNA with a component that is present in rate-limiting amounts and which is required for transcription initiation. It is unclear, however, how this mechanism might operate in the intact cell, as tRNA once processed is rapidly excluded from the nucleus, with no detectable back transport of mature tRNA from the cytoplasm[11]. We suggest that this human variant tRNA$_i^{Met}$ (either as a mature intranuclear species, or as a slowly processed precursor) might provide the intranuclear transcription aparatus with a means of sensing the output of the multigene tRNA$_i^{Met}$ family, perhaps by the mechanism suggested by the *in vitro* studies described.

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Inelastic neutron scattering analysis of hexokinase dynamics and its modification on binding of glucose

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The dynamical behaviour of proteins and its significance to protein function has become the subject of considerable experimental and theoretical investigation[1,2]. The existence of atomic motion about mean positions has been well established by the temperature dependence of X-ray diffraction[3] and it has long been recognized that considerable internal mobility is required in proteins to account for hydrogen exchange measurements[4]. However, only limited experimental information, mainly obtained by optical methods[5], is available on the frequencies associated with these fluctuations. Inelastic neutron scattering is a technique capable of yielding dynamical information about a system over a wide frequency range[6,7]. Recent advances in neutron spectrometers[8] and in the theoretical understanding of the dynamics of biological macromolecules have now made it both feasible and desirable to exploit this technique in the field of biophysics. To demonstrate the potential of the method, we report here data on the frequency distribution in solution of the enzyme hexokinase and its modification brought about on binding of its substrate glucose. The results lend support to the view that ligation induces a stiffening of the enzyme structure.

Inelastic neutron scattering arises when incident neutrons gain or lose energy as a result of interaction with the internal modes of motion of a sample. Techniques to measure these energy changes are routinely used to study the dynamics of solids and liquids[6]. In the case of organic molecules the signal is dominated by the incoherent scattering by the hydrogen nuclei and can be related to the frequency spectrum of the sample[7]. However, the application of this technique to proteins in solution is hampered by the necessarily low protein concentration (at best a few per cent) which means that all measured spectra are dominated by the scattering by the buffer. Use of buffers made from D_2O minimizes this background, but despite this, several previous attempts to measure the inelastic spectra of proteins in solution have suffered from insufficient statistical accuracy. This has been overcome by the time focusing time-of-flight spectrometer IN6[8] (see Fig. 1 legend) which has recently become available at the Institut Laue-Langevin, and enables measurements to be made with good counting statistics. Inelastic neutron scattering data on polycrystalline lysozyme have been reported previously[9].

We have chosen yeast hexokinase (molecular weight 50,000) for this investigation for the following reasons. Its structure is known from X-ray crystallography[10] and consists of two large domains with a deep cleft between them. A crystallographic study of an isozyme of hexokinase complexed with glucose has shown that in the complex the two domains have rotated so as

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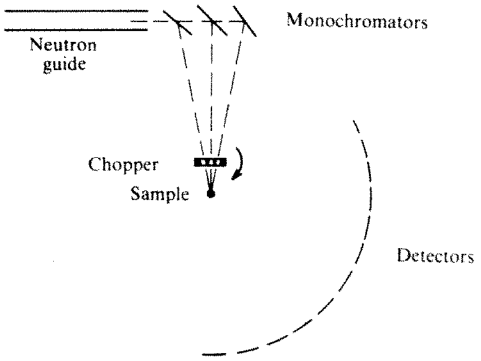

Fig. 1 Schematic diagram of time-focusing spectrometer IN6 viewed from above. Neutrons arriving through a guide tube ($3 \times 20\ \mathrm{cm}^2$) are monochromatized by a series of three pyrolytic graphite crystals with slightly different orientations. Each crystal reflects a different wavelength but all beams are focused on the sample. Pulses of neutrons are produced by a chopper which rotates about a vertical axis and is placed 40 cm before the sample. The chopper is designed so that the wavelength differences between the sub-beams are compensated by different times of chopping. In this way all elastically scattered neutrons arrive at the detector at the same time (time focusing). The instrument thus combines a high flux with a reasonable resolution (~ 0.15 meV or $1.2\ \mathrm{cm}^{-1}$ at the elastic peak). In the direction perpendicular to the diagram the neutron guide tube is 20 cm in height and a geometrical focusing of the beam in that direction is achieved by curved composite monochromators. The inelastically scattered spectrum is analysed by measuring the time of flight from the sample to a bank of detectors 247 cm away. The detectors were grouped to give 24 time-of-flight spectra corresponding to scattering angles between 10 and 115°. Each time-of-flight spectrum had 512 channels. Incident neutrons of wavelength 4.1 Å (4.87 meV) were used.

to trap the glucose in the cleft[11,12]. That this conformational change on binding of glucose actually occurs in solution has been confirmed by measuring the corresponding change in the radius of gyration using small-angle X-ray scattering[13]. The interest in making inelastic neutron scattering measurements on this system follows from the speculation that the binding of glucose in the cleft and resultant conformational change may modify the vibrational spectrum of hexokinase. In particular, relative motion of the two domains may be hindered on ligation. Furthermore, it has been argued that a change in the frequency spectrum of an enzyme on ligation may be significant in the thermodynamics of enzymatic reactions[14].

The experimental strategy was to collect data successively from the buffer, the hexokinase solution and the hexokinase saturated with glucose, all at 15 °C and in the same quartz cell. Figure 2 shows corrected and scaled spectra obtained by summing counts in the range of scattering angle between 84° and 110°. The spectra of the hexokinase solution and the buffer appear similar but the statistical accuracy is sufficiently high to show that the enzyme spectrum is significantly different in form from the unsubtracted spectrum. The signal from the protein is about 15% of the total. In subtracting the buffer a factor was introduced to take into account the lower buffer content of the protein solution. We verified that variation of this scaling factor by ±5% (a wider margin of error than the true error) did not significantly affect the form of the protein spectrum (in particular the peak around $50\ \mathrm{cm}^{-1}$). Thus, we believe that curves *c* and *d* in Fig. 2 are an accurate representation of the inelastic neutron spectra of hexokinase and hexokinase bound with glucose. Spectra were simultaneously measured at several scattering angles between 10° and 115°, and the angular dependence of the spectra can be summarized as follows. The intensity of the elastic peak of both the ligated and unligated spectra decrease roughly as $\exp(-0.89Q^2)$. The two main features of the inelastic spectrum—the peaks around 50 and $300\ \mathrm{cm}^{-1}$—behave differently. The former increases in intensity with angle while the latter maintains the same intensity and is hardly distinguishable as a distinct peak at a scattering angle of 97°. This different behaviour with angle of high and low frequency

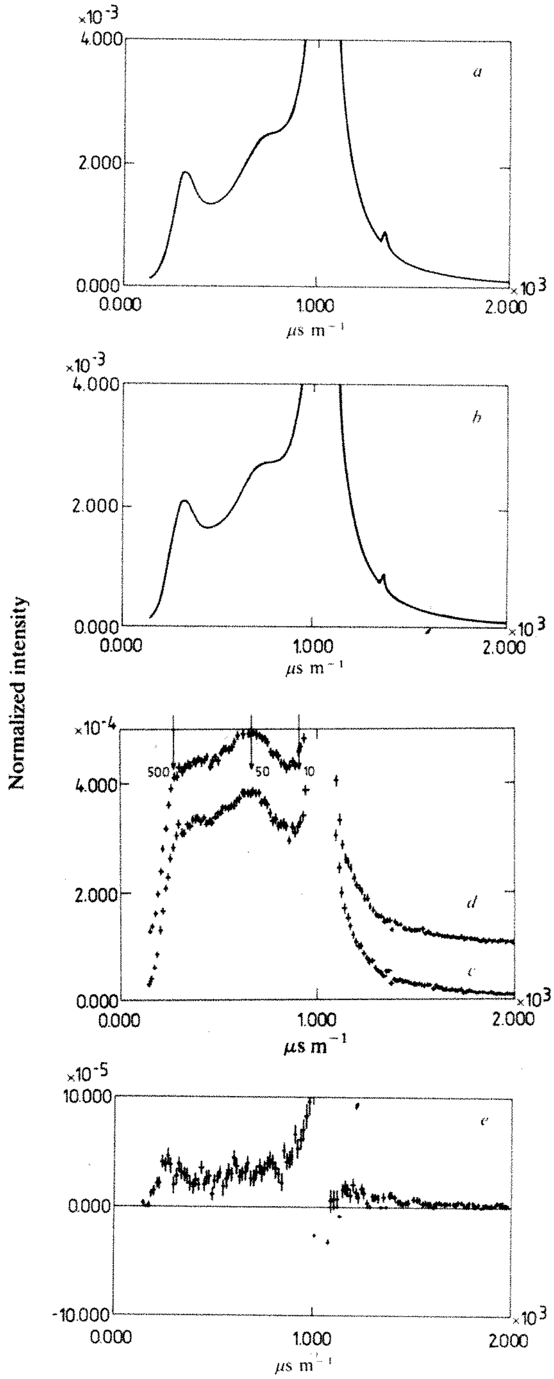

Fig 2 Time-of-flight spectra measured at a mean angle of 97° of buffer (*a*) and hexokinase solution (*b*). The ordinates have been scaled by the elastic peaks measured with a vanadium sample. The abscissa is in time-of-flight (TOF) units ($\mu\mathrm{s\ m}^{-1}$). Neutron energies are related to TOF by $E\ (\mathrm{cm}^{-1}) = 42.2 \times 10^6/(\mathrm{TOF})^2$. The sharp peak on the energy loss side of both spectra is due to parasitic Bragg scattering from an aluminium window. Subtraction of the buffer (and quartz cell) scattering gives the corrected spectra of hexokinase (*c*) and hexokinase bound with glucose (*d*) (curve shifted by 10^{-4}). Energy transfers of 10, 50 and $500\ \mathrm{cm}^{-1}$ are indicated by arrows. The difference spectrum obtained by subtracting *c* from *d* is shown in *e*. The error bars are derived from the counting statistics and show 1 s.d. from the nominal value. Hexokinase (Sigma lot 120F-8080) in crystalline suspension was redissolved in D_2O buffer containing 20 mM Tris, 0.1 M NaCl, 0.1 mM dithiothreitol, 0.1 mM sodium azide at $p\mathrm{D}(=p\mathrm{H})$ 8.5 and dialysed exhaustively against the same buffer. 0.8 ml of solution (previously concentrated to $45\ \mathrm{mg\ ml}^{-1}$) was then centrifuged at 16,000 r.p.m. for 5 min before being placed in a 1.5 mm thick quartz cell. The cell was set at 45° to the incident beam in the time-of-flight spectrometer and maintained at 15 °C. The sample chamber was maintained at a slight over pressure of helium throughout the experiment to avoid parasitic air scattering. To saturate the protein with its substrate, 1.8 mg of glucose per ml of solution was added, the glucose being in 50% (by weight) solution in the same D_2O buffer. The unexchanged protons of glucose increase the number of protons in the solution by about 3%. Spectra were accumulated for about 36 h each.

modes is consistent with the expected scattering by harmonic oscillators[7].

The dynamical modes of a protein should cover a wide range of different characteristic energies[2,5]. Some will be very similar to those found in small molecules, for example C—C bending and stretching modes and rotation of methyl groups. Characteristic energies for such local modes will be in the range 100–2,000 cm^{-1}. Others are collective modes specific to the secondary structure of proteins, for example longitudinal acoustic modes of helices with energies in the range 25–200 cm^{-1}. At still lower energies (1–50 cm^{-1}) 'breathing' modes as found in globular elastic bodies should occur, although the nature of these may be significantly modified by solvent damping[5]. Some of these low frequency modes may be directly linked with protein function, for example, the postulated hinge-bending mode in enzymes having a substrate binding cleft[15]. The inelastic spectra that we have measured extend up to more than 500 cm^{-1}, confirming the expected broad frequency spread of protein motions.

There are small but distinct differences between the spectra given by the enzyme with and without substrate bound (see Fig. 2*e*). First, there is an overall reduction of 5–10% in the intensity of inelastic scattering on glucose binding. This difference could be due *a priori* to a reduced number of protons in ligated hexokinase (resulting from a higher degree of exchange) or to a genuine modification of the enzyme dynamics. The first hypothesis is unlikely as the number of exchangeable protons is relatively small (20% of all protons) and is likely to decrease rather than increase with the tightening associated with substrate binding. Furthermore, neither the intensity nor the width of the elastic peak changes on ligation, suggesting that the total number of protons is unaltered. Thus, we conclude that there is a real modification of the dynamics of hexokinase on binding glucose. That the difference spectrum is positive suggests that the ligated enzyme has fewer and/or smaller amplitude vibrational modes, in accordance with the view that ligation induces a stiffening of the enzyme structure.

Second, Fig. 2*e* clearly shows that the shape of the difference profile differs from that of the total inelastic scattering by hexokinase. Thus, while ligation appears to affect both local and collective modes, different frequencies are affected to different extents. In particular, the low frequency modes ($<40\ \mathrm{cm}^{-1}$) are more markedly affected, as was also found to be the case on binding an inhibitor to lysozyme[9]. The hinge-bending mode of lysozyme has been calculated[15] to be between 5 and 15 cm^{-1}, and although no analogous calculations have been done for hexokinase, it is possible that the peak in the difference profile at 30 cm^{-1} corresponds to the disappearance of such a mode on closing of the cleft around the glucose substrate.

Intramolecular vibrational modes contribute to the enthalpy and entropy of a protein and it has been proposed that changes in the vibrational spectrum may be important in the thermodynamics of protein reactions[14]. Our results suggest that there is a reduction in the vibrational enthalpy of hexokinase on glucose binding. However, it has been shown recently that there is practically no overall change of enthalpy on binding of glucose[16]. This is not inconsistent with our finding, as a reduction in the enthalpy of the vibrational modes could be compensating for the burying of a charged group. Indeed, we note that such a group (Asp 189) is reported to be removed from contact with the solvent as a result of the conformational change[13]. In future experiments we aim to extract from the variation with angle of the inelastic scattering spectra the frequency distribution of vibrational modes of the sample[7]. This would allow estimation of the change in the vibrational contributions to the entropy and enthalpy on ligation.

In this exploratory application of inelastic neutron scattering, we have established that it is now feasible to use measurements of the frequency spectrum of proteins in solution to characterize the change in dynamical state associated with binding a substrate molecule. Clearly, further experiments of this kind will be needed to confirm the magnitude and significance of the observed changes. Several theoretical studies of protein fluctuations have been made by molecular dynamics simulations[2,15,17,18] and normal mode analysis[19], from which it is possible to predict inelastic neutron spectra. A more detailed interpretation of the inelastic neutron scattering spectrum of hexokinase would require careful comparison with the results of such calculations, although this is not yet practicable for such a large protein. Alternatively, one can envisage the use of selective labelling (for example, protonation of certain residues in an otherwise fully deuterated protein) to aid in assignment of particular modes. Thus, our results demonstrate the potential of inelastic neutron scattering for investigating the dynamic aspects of protein function and testing the theoretical understanding of protein dynamics.

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Errata

In the letter 'Pluripotent embryonic stem cell lines can be derived from t^{w5}/t^{w5} blastocysts' by T. Magnuson *et al.*, *Nature* **298**, 750–753 (1982), in Fig. 2 the lowest bands on the gels should be labelled 17 (kilobases). In line 3 of Fig. 2 legend $129(t^0/t^{w5})$ is incorrect and should read $129(t^0/t^{w12})$.

In the letter 'Potent new inhibitors of human renin' by M. Szelke *et al.*, *Nature* **299**, 555–557 (1982), structure 4 in Table 1 erroneously contains the superscript R over the Phe-Phe peptide bond.

Corrigenda

In the letter 'Direct imaging of travelling Rayleigh waves by stroboscopic X-ray topography' by R. W. Whatmore *et al.*, *Nature* **299**, 44–46 (1982), the references to 'half-wavelength' or '$\pi/2$' in Fig. 4 legend and in the relevant discussion in the text should read 'one wavelength' or 'π'.

In the News and Views article 'Monoclonal antibodies to human malignant melanoma' by R. A. Reisfeld, *Nature* **298**, 325–326 (1982), the names of two individuals who contributed considerably to the work described were omitted: Drs K. O. Lloyd and L. J. Old of the Memorial Sloan Kettering Cancer Center, New York.

The following note relates to the letter 'Do graded units of accretionary spheroids from the Barberton Greenstone Belt indicate Archaean deep water environment?' by I. G. Stanistreet *et al.*, *Nature* **293**, 280–284 (1981). Interested readers are referred to the work of T. Heinrichs, Lithostratigraphische Untersuchungen in der Fig Tree Gruppe des Barberton Greenstone Belt zwischen Umsoli und Lomati, *Gottinger Arb. Geol. Paläont.* **22**, 119pp (1980), which was not available to the authors during the preparation of the above manuscript.

BOOK REVIEWS

Ruskin then, science now

Michael Rodgers

Wiley: One Hundred and Seventy five Years of Publishing. By John Hammond Moore. Pp.279. ISBN 0-471-86082-4. (Wiley: 1982.) £14.75, $25.

COMPANY histories are not written primarily for the general reader and this one, celebrating 175 years of publishing at Wiley, is no exception. Nevertheless there is much here that those interested in books and publishing will appreciate.

In addition to journals, professional, research and reference works, Wiley's list contains many well-known science textbooks. Tracing the evolution of that particular species, the college textbook, provides the most interesting strand of this story, beginning in the 1940s when the United States government enacted its GI Bill of Rights. This entitled veterans of the Second World War to educational assistance, thus boosting the demand for college textbooks that had begun in the early 1940s, which in its turn arose from the armed services' large-scale provision of technical training. The bonanza for American textbook publishers came to an abrupt end in 1948, and not simply because the last of the ex-GIs had completed their college education. Publishers then were not over-concerned with such details as planning, cost accounting, and pricing policies; escalating costs were not being passed on to their customers. The boom fostered complacency, since little effort was required to satisfy students prepared to put up with dull, old-fashioned textbooks; their successors straight from school were more particular. A difficult period of adjustment lay ahead: a familiar pattern to those who, like me, entered publishing (in the UK) at the tail-end of the golden age of the 1960s.

For John Wiley & Sons there was the added irony that they had been steadily building a list of impressive titles in corporate management, yet they seemed unable to put this body of wisdom into practice. Their own solution to the general problem besetting the industry was to enrol four executives in the Harvard Business School's Advanced Management Programme in 1953. By the end of that year the company president, Bradford Wiley, was himself at Harvard to study modern business methods, returning

> enthusiastic, business-oriented, hardnosed, and determined to concentrate his full energies on the *financial* aspects of publishing.

No doubt it was an interesting time for Wiley employees as the new brooms returned.

Textbooks in the early days were the result of the buckshot approach: the publication of books with a broad appeal that *might* be adapted to specific course needs. In the 1950s the search for the customer still began after a textbook had been produced. Andrew Ford, a Wiley traveller of that era, notes that

> Editors relied on advisers, series editors, consultants, boards, and so on. Just look at our catalogs of those years. Nothing but one series after another.

This may produce a wry smile from some of my contemporaries in British publishing: we started out doing much the same thing, though 20 years later.

Ford, brought into the office as a junior editor in 1957, found

> an academic-like atmosphere where chief editors kept top schools to themselves like prizes for seniority and only grudgingly gave approval to manuscripts procured by a new employee.

The vital role of basic introductory textbooks in providing a reliable — and substantial — backlist income was yet to be fully appreciated. This was to change, and the 1960s saw a striking increase in Wiley's share of the college market. In 1961 their list contained 18 college titles with annual sales of 10,000 copies or more; in 1971 the figure had risen to 46.

How do the really successful textbooks actually begin life? It is a chancy business, for all the talk of planning, with the right author and the right editor coming together just when the market is ready for what they will eventually develop. We are given a fascinating glimpse of the genesis of Halliday and Resnick's *Physics for Students of Science and Engineering,* one of the most famous of science textbooks. Towards the end of 1954 one of Wiley's college editors, Robert B. Polhemus, happened to be visiting Resnick at the University of Pittsburg. A number of publishers had been urging Resnick and his departmental colleague Halliday to turn their lecture notes into a textbook. Polhemus noted this in his report to the New York office, continuing:

> The idea has blown hot and cold. Right now it's cold, and Halliday told me afterwards that he has definitely decided against doing such a book.

But they did and the rest, as they say, is history. Published in 1960, the parent text and its various abridgements and supplements have sold almost two million copies.

One year later Wiley merged with Interscience Publishers, the creation of two Europeans, Maurits Dekker and Eric S. Proskauer. Their original aim had been to provide a European-style publishing house in New York for émigré scientists in America. When the firm was established in 1940, German chemistry texts were used throughout the world: one of the first Interscience titles was an English translation of Karrer's *Organic Chemistry.* Thirty years later roles had been reversed, with the translation of Cotton and Wilkinson's *Inorganic Chemistry* becoming the standard text in German universities.

The predictable question concerning Wiley's future is posed on the penultimate page. In the 1980s they expect more expansion in the professional, medical and international groups than in the educational group: a reflection of an increasing demand for technical skills in the Third World coupled with a stable, or declining, college population in the developed countries. Of course it is the electronic revolution that will affect the company's long-term prospects, and although there are no specific forecasts, companies such as Wiley will doubtless have no real trouble in coping with the impact of electronics on reference sources generally. The best textbooks are a different matter. The experience of reading them could not be created by resorting to a screen, and their development will always need individuals. It is easy for Wiley to maintain that a future with an increasingly professional management, executives who refer to books as "product", sophisticated market research, and all the rest, will not stifle individual enterprise, but we look to their next decades' activity to prove the point.

Wiley's birthday volume is written in an earnest, respectful style, as befits an official history, but there are plenty of good stories and anecdotes about the life and times of a company that once published James Fenimore Cooper and John Ruskin. One appealing story concerns a railroad fan, Andrew Neilly, who joined Wiley as a traveller in order to see the nation by train, and for two years (1947–1949) he did just that. It must have been fun. And good background too, for he ended up as company president. ☐

Michael Rodgers, formerly with Oxford University Press and W.H. Freeman, is joining Longmans after a brief spell at Nature.

The protocol for arranging nature

Michael B. Usher

Multivariate Analysis in Community Ecology. By Hugh G. Gauch Jr. Pp.298. Hbk ISBN 0-521-23820-X; pbk ISBN 0-521-28240-3. (Cambridge University Press: 1982.) Hbk £20, $37.50; pbk £7.95, $14.95.

ECOLOGISTS collect multivariate data. Very rapidly the number of observations, each being the occurrence or abundance of one of a large number of species in one of a large number of quadrats, baffles the observer, who needs some kind of protocol for arranging and viewing the data. Multivariate analyses provide that protocol, but the diversity of possible methodologies can confuse all but the most numerate of ecologists.

In this new book, Hugh Gauch attempts to explain the methodology of multivariate analysis without delving into the mathematical or computational complexities. The introductory chapter deals with hand sorting: the sceptic only needs to look at Table 1.2 with 50 species recorded from 25 quadrats to see how even a small data set can look incomprehensible until it is re-arranged, as in Table 1.3.

Gauch makes the point that more objective methods for arranging all of the individual observations are required, and he devotes the remainder of the book to discussion of the three basic approaches to sorting ecological data of this type — direct gradient analysis, ordination and classification. Although the structure of each chapter differs, generally the rationale of the approach is described, the methods are explained in words rather than in mathematical symbols, a number of examples are quoted (illustrations lead to a geometrical interpretation of the results), some of the known problems are described, and finally there is a broad evaluation. It is these chapters which give the book its value.

There are two drawbacks, however, that prevent this being a complete textbook. First, if anyone actually wishes to undertake an analysis they will have to turn to another book for the recipe. True, there is a two-page appendix included on computer programs, but this is too brief to be a usable consumer's guide. Second, the text is very Cornell-orientated — perhaps not surprisingly, since Gauch is closely associated with the group which worked with the late R. H. Whittaker at that university. An example is the "horseshoe" problem, where their DECORANA method of tackling it is described but others are not considered (though to be fair the extensive catalogue of applications in Chapter 6 partly overcomes this problem).

Nonetheless, the clear explanation and the comparisons between the various approaches make the book a suitable text for the student or researcher who wishes — or has — to understand this field of quantitative ecology. As the author in general cleverly avoids the mathematical complications, the apparent simplicity may be beguiling. But I still plan to use this book as a background text when teaching multivariate methods. ☐

Michael B. Usher is a Senior Lecturer in the Department of Biology at the University of York.

Teaching machines

Margaret Boden

Intelligent Tutoring Systems. Edited by D. Sleeman and J.S. Brown. Pp.345. Hbk ISBN 0-12-648680-8. (Academic: 1982.) £19.20, $39.50.

INTELLIGENT tutoring systems (ITSs) are programmed teaching-aids grounded in artificial intelligence (AI). They differ radically from familiar types of computer-assisted-instruction (CAI). Many of the latter merely present repetitive "drill-and-practice" exercises; others can vary their response with the student's level of understanding by means of branched programs with predefined choice-points, but only to a very limited degree. ITSs are considerably more flexible, and better able to adapt to the idiosyncracies of a student's thinking.

This flexibility derives from the fact that ITSs contain complex computational models of students' knowledge and thinking. These control the questions and responses that an ITS presents to the student, and generate its diagnoses of students' mistakes. Unlike traditional CAI, the emphasis of ITSs is less on facts than on ways of thinking about a problem-domain; they function not so much by presenting "correct" examples of solutions as by providing relevant hints or questions, and — significantly — by diagnosing the thought-strategies behind the student's mistakes so as to guide the tutorial response.

Such diagnosis requires an understanding (in explicit, programmed, form) of the variations in cognitive structure and inference that underlie different sorts of (non-random) mistake. Computer science has a word for such mistakes — "bugs". A bug is a precisely definable and relatively systematic erroneous variation of a correct procedure (this definition, though, optimistically implies an underlying correctness). Much work on ITSs is focused on identifying and using bugs in the student's thinking.

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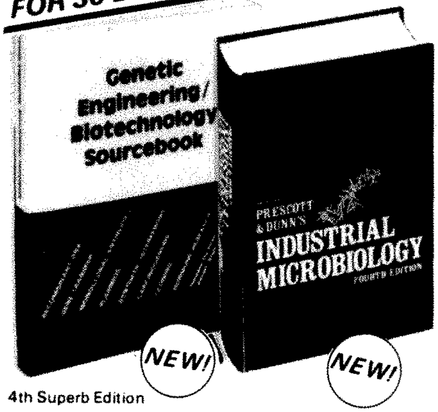
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nosing the (nearly one hundred) different bugs that can explain a student's errors in doing subtraction. Its authors have provided a precise notation for describing these, and are also developing a "generative" theory of bugs — that is, one that can be applied to a particular (correct) procedural skill to generate all the bugs actually observed, and no others. This theory aims also to explain the pattern of bugs observed, so that (for example) two superficially different mistakes in subtraction can be understood as two variations of the same underlying difficulty in the student's mind. The ITS thus embodies a psychological theory predicting the detailed errors observed during learning.

But this program, like most of the other ITSs described in Sleeman and Brown's volume, could not be let loose in our schools tomorrow. This is partly because its psychological theory (like all current theories of learning) is inadequate to capture the full complexity of learning, and partly because AI cannot yet provide a "friendly" computing environment such that an untrained person (child or teacher) can use it. Given a specially-trained teacher, however, it can be used in the classroom to help identify precisely what is going awry in the children's thinking.

The complexity of the computational models within ITSs is such that they deal only with limited topics, as opposed to entire curricular courses (a further contrast between ITSs and CAI). Examples described in the book (which is edited by two pioneers in the field) include medical diagnosis of specific kinds, faults in electronic circuits, interpretation of magnetic resonance spectra and various mathematical topics. But the editors suggest that many of these forms of thinking are generally useful outside these particular applications — perhaps ITSs are the new Latin?

Sleeman and Brown's useful collection summarizes the state-of-the-art, and highlights problems as well as promises. They admit that current ITSs are excessively *ad hoc*, reflecting unprincipled intuitions about how to control their behaviour rather than well-founded psychological and computational theories about teaching–learning strategies. As well as improvements in AI techniques (for handling natural language dialogue, for example) we need more experimental investigation of how to transform students' misconceptions into constructive learning experiences. But ITSs could help direct the relevant research. One can thus endorse the editors' words:

> We hope that educational theorists will find the explicit formulation of tutoring, explanation and diagnostic processes inherent in ITSs a test bed for developing more precise theories of teaching and learning. ☐

Margaret A. Boden is Professor of Philosophy and Psychology at the University of Sussex.

Inside cytoplasm for outsiders

Harriet Harris

Organization of the Cytoplasm, Parts 1 and 2. *Cold Spring Harbor Symposia on Quantitative Biology*, Vol. XLVI. Pp.1,047. ISBN 0-87969-045-3. (Cold Spring Harbor Laboratory: 1982.) $130 (US), $156 (elsewhere).

One of the principal goals of cell biology is to describe the functions and behaviour of the living cell in terms of its molecular components. Even with the objective restricted to an understanding of the cytoplasm, however, the complexity of function and the number of constituent organelles and macromolecular components is daunting. Nonetheless the progress with certain specialized systems (such as eukaryotic flagellae or intestinal microvilli) towards the correlation of biochemistry, structural organization and dynamic function gives hope that the ultimate goal can be achieved.

The symposium on which these volumes are based brought together a wide diversity of topics, ranging from properties of bulk cytoplasm to details of molecular interactions. It would be fair to comment that relatively few of the contributors to the books might be described as studying cytoplasm *per se*, and it appears that one aim of the two-volume work is to stimulate a sense of common purpose between researchers in fields as distinct as, for example, membranes and microtubules.

The behaviour of living cytoplasm must provide the essential framework within which to view both ultrastructural and biochemical results. This field is being greatly stimulated by new techniques in light microscopy, sometimes in combination with the microinjection of foreign substances into the cytoplasm. These yield information on the movement of macromolecules and organelles through the cytoplasm, or may detect changes in important regulatory factors such as *p*H and calcium ion concentration. Such developments are still in their infancy, but offer the exciting possibility of enabling us to describe actual events in the cytoplasm, which is a vital step on the way to elucidating by what mechanisms such events occur.

A fruitful approach to mapping the spatial organization of the cytoplasm has been to localize known proteins within fixed cells by means of specific antibodies, for example, by immunofluorescence. Such techniques have been nowhere more productive than in the extensive characterization of three sets of cytoskeletal filaments — microtubules, intermediate (100Å) filaments and microfilaments — all of which are well represented in these volumes. A description of the location and composition of a structure, however, is not necessarily the key to comprehending its function, and an outstanding question on the cytoskeleton concerns the role of intermediate filaments. This problem is being tackled by microinjection of filament-specific antibodies to selectively dissociate the networks within living cells, an experimental approach which holds promise as a general method for probing elusive functions. Often the most useful data on the static organization of macromolecules are obtained with simplified systems. Two striking examples are the packing of actin filaments in stereocilia and the linkage of the cytoskeleton to membranes in erythrocytes, both of which provide general models applicable to less specialized cell types.

Another theme of the symposium, reflected in the books, concerned the mechanisms by which cytoplasmic order can be maintained in complex, multicomponent, dynamic systems. This is a speculative area and fertile ground for hypothetical models, but some highly productive experimental systems are also described. Protein-sorting problems are discussed by several authors, in a variety of contexts; these include selective insertion of proteins into or through membranes on synthesis, synthesis and expression of cell surface or secretory proteins, and selective uptake at the surface, all processes which may occur rapidly with continuous turnover and where high specificity is essential to deliver the proteins to their correct destinations. Other cytoplasmic components which may be in a highly dynamic state are the microfilaments and microtubules, where these form transient assemblies, such as the mitotic spindle. Assembly must be under rigorous control, since the correct location and polarity of filaments is essential in determining function. Current data indicate that order may be achieved by discrete organizing centres for filament growth, but these would only be effective in conjunction with the suppression of spontaneous, unregulated filament formation. A wealth of data from *in vitro* biochemical studies of filaments and their associated proteins generally support such a model, but proof of the effective modes of control *in vivo* will be more difficult to achieve.

What are the physiological switches signalling changes in cytoplasmic behaviour? Calcium, acting both directly and by calmodulin-mediated processes, including phosphorylation, is heavily implicated, as are cyclic AMP-dependent and other kinases. The space devoted to regulation in the books is disappointingly small, perhaps because the areas of ignorance are still too large for meaningful questions to be asked.

The attempt of the editors to unite a range of subjects under the single heading of cytoplasmic organization is timely, but not wholly successful. Fully comprehensive coverage of such a large field is not to be expected, even in a thousand pages, but the balance between different topics is in places very uneven. Only a handful of articles, for example, make any reference to the soluble components of the cytoplasm, but nearly half of the books (surely too much) is devoted to cytoplasmic filaments. These volumes do however contain a wealth of interesting material, while the breadth of ground covered makes them especially valuable to more general readers, or to cell biologists exploring areas peripheral to their own. □

Harriet Harris is at the Laboratory of Molecular Biology, Cambridge, UK.

All of the Araneae pulled together

J.L. Cloudsley-Thompson

Biology of Spiders. By R.F. Foelix. Pp.306. ISBN 0-674-07431-9. (Harvard University Press: 1982.) $35, £21.

ALTHOUGH there are some recent and quite excellent books on the ecology and natural history of arachnids in general and of spiders in particular, no new attempt has been made for over half a century to provide a comprehensive synthesis of the general biology of these fascinating creatures. The book under review, the original version of which appeared in 1979 as *Biologie der Spinnen* (published by Georg Thieme Verlag, Stuttgart), is an attempt to pull together the various facets of a spider's life. It is not a condensed handbook or reference book so much as an advanced introduction to the group.

Spiders are found throughout the world and, according to Rainer Foelix, "have conquered all ecological environments, with perhaps the single exception of the air": and, one might add, of the seas. The biology of freshwater spiders (*Argyroneta aquatica, Dolomedes* spp. etc.) is discussed in some detail, but little mention has been made of marine littoral spiders, although these do occur as well. It is, in truth, no exaggeration to say that all possible ecological niches on land have been occupied, including rather barren environments such as sand dunes, tidal zones or mountain tops. The distribution of spiders, extending even to oceanic islands, can be explained by the ability of the young of many species as well as of adult Linyphiidae, to float through the air, ballooning on their own threads. That spiders cannot bridge really large distances by aeronautical means, however, is indicated by the fact that East Africa and Madagascar are only 400 km apart, yet the spider fauna of each is quite different.

The interactions between spiders and their environments have been investigated systematically only within the past few decades. During this time, however, information about the ecology of spiders has burgeoned to such an extent that only a few selected themes have been discussed in the chapter on ecology. These include distribution, habitats, prey, enemies, thermoregulation, overwintering, camouflage and mimicry, communication and social behaviour. Other chapters are concerned with functional anatomy, metabolism, neurobiology, spider webs, locomotion and prey capture, reproduction, development, phylogeny and systematics.

The book is well illustrated with photographs and line drawings. It is interestingly written, and the author has managed to convey to the reader some of his own obvious enthusiasm for the subject. He cannot fail to have engendered further interest in spiders, thereby militating against the neglect they have so long been accorded and which he himself so deplores.

J.L. Cloudsley-Thompson is Professor of Zoology at Birkbeck College, University of London.

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Tectonics: moving with the times

Janet V. Watson

Plate Tectonics and Crustal Evolution, 2nd Edn. By Kent C. Condie. Pp.310. Hbk ISBN 0-08-028076-5; pbk ISBN 0-08-028075-7. (Pergamon: 1982.) Hbk £32.50, $65; pbk £13.10, $29.50.

SO MANY books have been written in the aftermath of the plate tectonic upheaval that any new arrival is bound to encounter some resistance. Kent Condie's volume is a second edition, issued six years after the original, and can therefore be said to have already justified its existence.

Has the new edition moved with the times? Although text, figures and tectonic map have been updated with respect to many details, my impression is that the book retains not only the merits but also the defects of its predecessor. Kent Condie is distinguished for his work on Precambrian magmatic assemblages, and his treatment of the Precambrian magmatic provinces and of igneous phenomena generally is thorough and effective. He is successful in showing how geophysical and geochemical data can contribute to the understanding of geological problems and he has been unusually courageous in his attempts to elicit a general picture from the scrappy and often contradictory evidence about the deep structure of the crust and mantle. The numbers assigned to such values as the average thicknesses of crust in different types of province make good Aunt Sallys for the knowledgeable, though the less-experienced reader might have been better served if a range rather than an average had been quoted.

The inequalities of standard of treatment seem more apparent in this second edition than they did in 1976. In particular the disparity between the sophisticated discussion of magmatic phenomena and the cursory treatment of sedimentary basins has become more glaring. The author justifies the omissions on the reasonable grounds of lack of space and on the need to avoid overlap with other published works. Nonetheless, plate tectonics without the sedimentation story is Hamlet with no more than half the Prince of Denmark.

I have one final personal regret which concerns the otherwise welcome world tectonic map which is contained in a pocket at the back of the book. Maps are not only valuable sources of information, they can also be objects of beauty that kindle the imagination and stimulate the ininitiated and the expert alike; this one succeeds in its first role but is sadly deficient in the second. □

Janet Watson is a Professor in the Department of Geology at Imperial College, University of London.

ANNOUNCEMENTS

Awards

The 1982 Kone Award has been awarded by the Association of Clinical Biochemists to **Professor Poul Astrup** (Copenhagen) for his contribution to clinical chemistry.

The winners of Columbia University's 1982 Louisa Gross Horwitz Prize are **Barbara McClintock** (Carnegie Institution's Genetic Research Unit at Cold Spring Harbor) and **Susumu Tonegawa** (Center for Cancer Research and the Department of Biology at the Massachusetts Institute of Technology).

The Lita Annenberg Hazen Award and Fellowship Grant aims to recognize major achievement and superior potential and to foster a greater commitment by physicians to clinical research. A prize of $50,000 (tax free) will be awarded to the physician-investigator or team of physicians for current and published work, that is of evident superiority and importance. A companion grant of $50,000 will be given to the investigator's institution for the support of a Research Fellow or Fellows whom the Award winner will select as associates. Further details from Mount Sinai School of Medicine of the City University of New York, Office of the Dean, 1 Gustave L. Levy Place, New York, New York 10029, USA.

Applications for training fellowships in 1983–84 are invited from junior scientists wishing to be trained in those aspects of cancer research related to the International Agency for Research on Cancer's programme: epidemiology, biostatistics and environmental carcinogenesis — both chemical and viral. Applicants should be engaged in research in medical or allied sciences and intend to pursue a career in cancer research. Fellowships are awarded for one year and are tenable at the Agency or in another suitable institution abroad. Further details from: the Head of the Research Training and Liaison Programme, International Agency for Research on Cancer, 150 cours Albert-Thomas, 69372 Lyon Cedex 08, France.

The National Physical Laboratory is to set up an Awards Scheme aimed at furthering knowledge and understanding of metrology. In 1983 single-payment awards of up to £5,000 will be made to individual scientists on the full-time academic staff of UK universities and polytechnics, who submit the best ideas and proposals for research and development work likely to lead to a major advancement in measurement. Applications should reach Dr K.C. Shotton, Secretary, NPL Metrology Awards Scheme, National Physical Laboratory, Teddington, Middlesex, UK, by 31 December 1982.

The Institution of Mechanical Engineers and Esso announce the Tribology Award 1982/83. A £300 prize will be given for an essay on industrial aspects of lubrication, friction or wear, submitted by 31 March 1983. Anyone under 35 engaged in industry or on a sandwich course may enter. Further details from. Mr J.H. Cole, Divisions Co-ordinator, The Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London SW1, UK.

If you work in a university outside the UK, or in a polytechnic, college or school anywhere and your work is held up for lack of modest funding, the RSC Research Fund may be able to help you. Further details from: Mrs E.S. Wellingham, The Royal Society of Chemistry, Burlington House, London W1, UK.

Appointments

Mr Roland List (Canada) has been appointed Deputy Secretary General of the World Meteorological Organization (WMO) succeeding Raymond Jean Schneider (Switzerland) who has retired.

The new board of governors of the International Atomic Energy (IAEA) have elected the governor from Czechoslovakia, **Ambassador Emil Kebluser** to be Chairman from 1982 to 1983. The vice-chairmen are: **Luigi Noe** (Italy) and **Adolfo R. Taylhardat** (Venezuela). **Bernard Bechetoille** (France) is the new director of the Division of Budget and Finance.

The following appointments have been made within CSIRO: **Dr David Smiles** has become chief of the Division of Soils, based in Canberra; **Dr Don Gibson** is chief of the Division of Energy Technology in Melbourne; **Dr David Mitchell** is officer in charge of the Centre for Irrigation Research at Griffith in New South Wales; **Dr Alan Reid** is chief of the Division of Mineral Engineering in Melbourne.

Sir Keith Joseph, Secretary of State for Education and Science, has appointed three new members to the Natural Environment Research Council: **Lord Cranbrook, Professor Ronald Edwards** and **Professor John Simpson.** Reappointed are **Professor William Stewart, Mr Hugh Fish** and **Professor Bernard Leake.**

J. Mark Danley has been appointed Group Leader for Education and Training at BioSciences Information Service (BIOSIS).

Dr I. Kelsey Fry is the new president of the British Institute of Radiology 1982–83.

Mr John Banks has been appointed president of the Institution of Electrical Engineers (IEE). The retiring President is Sir Francis Tombs.

Dr Donald J. Reis has been chosen as the first George C. Cotzias Distinguished Professor of Neurology at Cornell University Medical College.

The French Minister of National Education has conferred the title of Doctor Honoris Causa of the University of Nancy on **Prof. A.R. Ubbelohde** (Imperial College, University of London).

The governing body of the Rowett Research Institute has appointed **Dr Philip James** to the post of director in succession to Sir Kenneth Blaxter.

Derek M. Lambert (Cyril Blumfield & Partners) has been appointed president of the Concrete Society.

Martin C. Steele, formerly in charge of the semiconductor electronics group at the General Motors Research Laboratory, has been appointed acting director of the Institute for Amorphous Studies, Michigan.

George A.M. Cross, formerly at the Wellcome Research Laboratories (Beckenham, UK) has been appointed a professor at The Rockefeller University to head a new laboratory of molecular parasitology.

Dr C.W. Davidson (Department of Electrical and Electronic Engineering at Heriot-Watt University) is the new chairman of the Institution of Electrical Engineers' Science, Education and Technology Division.

Meetings

7 December, **Plant Cell Culture,** London (S. Whittaker, Oyez Scientific and Technical Services Ltd, Bath House, Holborn Viaduct, London EC1, UK).

15-17 December, **Annual Meeting of the British Medical Ultrasound Society,** Sheffield (British Institute of Radiology, Institute House, 36 Portland Place, London W1, UK).

20-22 December, **Biochemistry of Carcinogenesis,** London (The Biochemical Society, 7 Warwick Court, High Holborn, London WC1, UK).

20-22 December, **The Detection and Measurement of Hazardous Substances in the Atmosphere,** London (Dr J.F. Gibson, The Royal Society of Chemistry, Burlington House, London W1, UK).

10-13 January 1983, **Development of the Vascular System,** London (The Ciba Foundation, 41 Portland Place, London W1, UK).

17-21 January, **CIMO Working Group on Upper-Air Technology Basic to User Needs**, Geneva (World Weather Watch Department, World Meteorological Office, Geneva, Switzerland).

25 January, **Radiological Protection — Future Research Requirements**, London (Prof. J.H. Martin, Dept of Medical Biophysics, Blackness Laboratory, University of Dundee, Dundee, UK).

7-11 February, **Water Hyacinth**, Hyderbad (Dr G. Thyagerajan, Regional Research Laboratory, Hyderabad 500 009, India).

13-18 February, **Recent Advances in Bone Marrow Transplantation**, Utah (UCLA Symposia, Molecular Biology Institute, University of California, Los Angeles, California 90024, USA).

16 February, **New Radiopharmaceuticals and their Clinical Applications**, London (The British Institute of Radiology, Institute House, 36 Portland Place, London W1, UK).

21-23 February, **Diet and Nutrition**, Tel Aviv (The Secretariat, International Conference on Diet and Nutrition, PO Box 29784, Tel Aviv, 61297, Israel).

21-25 February, **Cooperative Education**, Melbourne (The Chairman, World Conference on Cooperative Education, PO Box 218, Hawthorn, Victoria 3122, Australia).

1-4 March, **Space Physiology**, Toulouse (CNES, Department des Affaires Universitaires, 18 Ave Edouard-Belin, 31055 Toulouse Cedex, France).

21 February — 4 March, **Commission for Agricultural Meteorology**, Geneva (World Climate Programme Department, World Meteorological Organization, Geneva, Switzerland).

7-12 March, **Analytical Chemistry and Applied Spectroscopy**, Atlantic City (Pittsburgh Conference, 437 Donald Rd, Pittsburgh, Pennsylvania 15235, USA).

8-11 March, **Nickel in the Human Environment**, Lyon (Dr W. Davis, IARC, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France).

9 March, **Digital Radiography**, London (The British Institute of Radiology, Institute House, 36 Portland Place, London W1, UK).

13-17 March, **Microlithography**, California (SPIE, PO Box 10, Bellingham, Washington 98227-0010, USA).

14-16 March, **Biomedical Research**, Heidelberg (Charles River Foundation, PO Box 430, Wilmington, Massachusetts 01887, USA).

14-17 March, **Microbiological Quality Assurance in Industry**, Brighton (Scientific Symposia Ltd, 33-35 Bowling Green Lane, London EC1, UK).

14-17 March, **Poliomyelitis Control**, Washington DC (Dr E.C. Chamberlayne, Fogarty International Center, Building 16A Room 205, National Institutes of Health, Bethesda, Maryland 20205, USA).

14-18 March, **Reactions and Intermediates in Nitrogen Fixation Processes**, Brighton (EUCHEM Conference, ARC Unit of Nitrogen Fixation, University of Sussex, Brighton, UK).

14-18 March, **Informal Coordination Meeting on the Use of INMARSAT**, Geneva (World Weather Watch Department, World Meteorological Organization, Geneva, Switzerland).

27-30 March, **1st European Symposium on Radiopharmacy and Radiopharmaceuticals**, Elsnore (Isotope Pharmacy, Frederikssundsvej 378, DK 2700 Copenhagen/Bronsho, Denmark).

21-25 March, **Annual American Society for Neurochemistry Meeting**, Honolulu (Dr G.R. Dutton, College of Medicine, University of Iowa, Iowa 52242, USA).

25-28 March, **British Society for Cell Biology**, Liverpool (Local Meeting Secretary, Dept of Medical Cell Biology, Liverpool University, UK).

26 March-1st April, **Gene Expression**, Park City (UCLA Symposia, Molecular Biology Institute, University of California, Los Angeles, California 90024, USA).

New ventures

The Arab Bureau of Education for the Gulf States has started publishing a new journal, entitled *The Arab Gulf Journal of Scientific Research* which will be devoted to research in basic and applied sciences. All communications should be directed to: Managing Editor, Arab Bureau of Education for the Gulf States, PO Box 3908, Riyadh, Saudi Arabia.

During the 18th General Assembly of the International Astronomical Union, a new IAU commission with the title: 'Commission 51 — Search for Extraterrestrial Life' was approved. The objectives will include: the search for planets around other stars; the search for radio transmissions, intentional or unintentional, of extraterrestrial origin; the search for biologically relevant interstellar molecules and the study of their formation processes; detection methods for potential spectroscopic evidence of biological activity; and finally the coordination of efforts in all these areas at the international level. Prof. Michael D. Papagiannis was elected President of the new commission and all IAU members who would like to join the new commission are requested to write to him at the Dept of Astronomy, Boston University, Boston, Massachusetts 02215, USA.

Following the First International Symposium on Radiation Physics, ISRP-1 (Calcutta 1974), interest has been expressed in forming an International Radiation Physics Society (IRPS) which could serve as the primary sponsor for further symposia in this interdisciplinary subject area. A protem committee has now been elected to explore and to take the necessary actions to form such a society. The primary objective of which society would be the global exchange and integration of scientific information pertaining to radiation physics, with emphasis on (1) research, theoretical and experimental, in the field of radiation physics, (2) investigations of the physical aspects of the interactions of radiation with living systems, (3) education in radiation physics and (4) utilization of radiation for peaceful purposes. Further information from Prof. D.F. Jackson, University of Surrey, Guildford, UK.

A new scientific society, the American Society for Virology, has been officially inauguarated with the first of what will be annual meetings. W.K. Joklik of Duke University, was elected president of the society. The major purpose of the society will be the organization of annual meetings; the 1983 meeting being scheduled for Michigan State University next summer.

The International Society of Cryptozoology aims to be a focal point for the investigation, analysis, publication, and discussion of all matters related to animals of unexpected form or size, or unexpected occurrence in time or space. The society will promote the scientific examination of all evidence related to these matters. Although the society is primarily intended for biological scientists, membership is open to all interested persons. The society will publish both a quarterly newsletter and a scholarly, refereed journal, entitled *Cryptozoology*, which will appear once a year. Further information can be obtained from J. Richard Greenwell, International Society of Cryptozoology, PO Box 43070, Tucson, Arizona 85733, USA.

The Agricultural Research Council is to establish an Animal Breeding Liaison Group within the Edinburgh School of Agriculture, under the leadership of Prof. J.W.B. King. Its role will be to identify problems of current concern within the animal breeding industry and to promote the rapid uptake of research into farming practice. The group will liaise closely with other research establishments and relevant industrial organizations. Further information from M.L.S. Delaney, Agricultural Research Council, 160 Great Portland St, London W1, UK.

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Patching up the pipeline quarrel

The United States and Western European governments are trying to settle their quarrel about the Siberian pipeline. Their most important task is to find a way of rewriting the strategic embargo.

The great conflict between the United States and several industrial states in Western Europe about the pipeline intended to carry natural gas from the Soviet Union to the West seems to have entered a new phase — one in which statesmen wish they had behaved differently. Their immediate difficulty is that of knowing how best to retreat from positions they have established by earlier declarations that the pipeline dispute entails issues of principle that cannot be given up. The result is that a number of manufacturers that have supplied compressors for the Soviet pipeline are still on a kind of US blacklist, denied supplies of virtually anything from across the Atlantic. This circumstance is manifestly inequitable, if only because the dispute concerns not the companies themselves, which would have had to break binding contracts with the Soviet Union to fall in with the Regan Administration's wishes, but their national governments. By now, there are obvious signs that the United States would willingly climb down from the hot-tempered position it assumed in the summer, but that it must have in return an agreement with Western Europe on trade with Eastern Europe and the Soviet Union. On what grounds can such an agreement be drawn?

As with other disputes, confrontation is more easily arranged than mollifying settlement. Both sides, the United States and West European governments, are affronted by the way in which their quarrel has blown up. In Europe, there is a widespread view that Mr Reagan should have said more plainly how strongly he and his colleagues felt about European participation in the pipeline project when they had a chance to speak their minds at the Versailles summit meeting last June. The United States, by contrast, claims to have been injured by the French government, which is accused of having misled it, but that complaint is insubstantial. The French government has made no bones about its determination to help with the pipeline ever since M. Mitterrand's election as President. It would have been especially hard to change course at about the time that the Soviet Union, under the terms of an agreement with France on scientific and technical collaboration, was planning to put the first Frenchman into an orbit about the Earth.

The difficulty in deciding where to go from here stems from the complexity of the underlying issues, and of the disagreements of principle that attend them. The most serious difficulty is that of dealing with the philosophical conundrum of knowing what the economic relationship should be between militarily hostile powers, the Soviet Union on the one hand, the United States and most of Western Europe on the other. In the old days, nobody bothered very much. France and Britain traded their way through the Napoleonic wars without much difficulty. The discovery that international hostility has economic dimensions dates only from the First World War, and from the recognition then that nation states could be brought to their knees by being physically deprived of goods, commodities and manufactures. Since the Second World War, this doctrine has been elaborated, perhaps further than logic will allow. At various times, both the United States and Western European governments have sought to distinguish between different kinds of goods: commodities embodying high-technology (Cray computers) have been on the West's strategic embargo list, but other materials (wheat, or less advanced computers) have been free.

One of the novel considerations that colour US policy on relations with the Soviet Union is the recent recognition that the United States and Western Europe no longer have a monopoly of high technology (there is, after all, Japan), so that agreements that enable the Soviet Union to earn hard currency may make possible the purchase of high technology from outside the old magic circle. (This is the sense in which President Reagan is right to assert that his administration is not being inconsistent in agreeing that the farmers of the Middle West should continue to supply cereals to the Soviet Union, for that is a way of sucking up convertible currency, not for increasing the supply of it.) That calculation cannot, however, be central, even in Washington. If the pipeline does generate for the Soviet Union a steady cash flow in convertible currency, the result will most probably be an increase of general purchases from the West, wheat, meat, dietary fats at the top of the list but other simple manufactures as well. Especially because it is now plain that the Soviet Union will not have hard currency running out of its ears even when the pipeline is complete, and so will not be able to lavish support on its ambiguous (and dubious?) allies around the world, Washington's hard currency objection to the pipeline agreement is unconvincing.

Washington's argument can indeed be stood on its head. Detente in the sense of the early 1970s may be dead (with Afghanistan and, later, Poland), but the old struggle between two conflicting ideologies remains. So what better means of persuading Soviet governments and their dependants of the ideology that Adam Smith has been preaching for the past two centuries than to engage the Soviet Union in honest trade? Conversely, will not trade that is too tightly regulated put at risk whatever hope survives that the two systems may one day come to a durable understanding? In short, if the United States Administration is looking not for an agreement that it can present to Congress as a diplomatic triumph but for a basis for future understanding with Western Europe, it should stop complaining about the hard currency the Soviet Union will earn from building the pipeline.

The more substantial objection to European participation in that project is the complaint that Western Europe will become strategically dangerously dependent on the Soviet Union if it becomes too substantial a customer for Siberian gas. There is no denying that it is anomalous that governments planning how and where to give hospitality to a new generation of US missiles aimed at the Soviet Union should simultaneously be planning to make themselves to some degree dependent industrially on a natural product supplied from the Soviet Union. Quantitatively, the dependence of Western Europe on the pipeline will be marginal, but its symbolic significance cannot be ignored. Western Europe might therefore without dishonour agree with the Reagan Administration that participation in a second pipeline project could judiciously be postponed.

The much more difficult decision is how the West at large should regulate the transfer of high technology to the Soviet Union. The strategic embargo list is itself as anomalous as the central assumption on which it is repeatedly drawn up — that there is a hierarchy among different kinds of know-how, and that it is possible by means of, say, a strict embargo on trade in bicycles with gear-shifts to deny to some trading partner the opportunity ever to improve the performance of the direct-drive bicycles that he is allowed to buy in bulk. The same goes for mousetraps but

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also computing machinery. The moral is that the Reagan Administration should now be bending its efforts to devising a system for a strategic embargo in which forbidden exports are classified not by type and catalogue number but by the effort in research and development that would be required to turn them into militarily useful weapons. On past experience, the United States would be chancing its arm by aiming at an advantage in time of three years or thereabouts. Does the present administration have the courage to acknowledge that that is about the best that can be hoped for?

Blow cold, blow hot

A relaxation of austerity has benefited the British scientific enterprise; but strings are attached.

The conclusion that the British Secretary of State for Education and Science, Sir Keith Joseph, may in reality be a better minister than he likes it to be thought emerged earlier this week from the Chancellor of the Exchequer's forward look at public spending in the three years immediately ahead. Sir Keith seems to have won from the Treasury an extra £2 million, or 0.4 per cent, of the budgets of the research councils in the financial year immediately ahead (beginning on 1 April next year). But since, unexpectedly, it has become known that an extra £4 million will be spent next year on the British Antarctic Survey, there is no way of telling whether the Natural Environment Research Council (the survey's sponsor) will be deprived of a further £2 million. Plainly, all departments of state in the British government are still fighting the Falklands war (which is not to say that the Antarctic survey will not put the extra funds fallen into its lap to good use).

The more valuable component of the science budget for next year, as now amended by the British Treasury, is what was being said earlier this week about the special support to enable universities to recruit young people to academic posts. Sir Keith has already stolen £6 million from the budget of the Social Science Research Council for this purpose, but when that will become available is to some extent in the hands of the research council itself. Now the intention is that up to £10 million should be spent on the recruitment of talented people (mostly in the sciences) to posts in the universities. For the time being, the fact that it is by no means clear how the extra money will be spent will not shade the joy with which university campuses will be suffused. For the best part of a decade, British universities have been unable to recruit to their teaching staffs the talented people whom, in other times, they would have snapped up. Even the most secure among older academics now acknowledge their need of "young blood". So, it should be said, they should have done some time ago.

Apart from the uncertainty about the source of this extra money, and its duration, the only difficulty in what the Department of Education and Science was saying this week after the Chancellor's declaration was that some of the extra money to be channelled towards the universities will be spent on courses in information technology. Apparently Sir Keith Joseph has been listening to Mr Kenneth Baker, the minister of state at the Department of Industry with special responsibility in this field. While Mr Baker is dangerously oversold on this government's plan to install cable television in Britain any day now (see *Nature* 28 October, p.765), he is right to be plugging the virtues of the technology of information. The open question, which Sir Keith Joseph should be brooding about, is whether it is right (let alone proper) for the Department of Education and Science to be telling universities that they can have extra money provided that they toe some ministerially determined line. In their hearts they know they should resist, and collectively refuse the money. In their boots, they know there are enough among them who will accept to undermine whatever high-minded position the high-minded among them might adopt. In short, the universities are being told that there will be graduate courses on information technology even if individual universities see no need of them. Sir Keith's success in persuading the Chancellor of the Exchequer may, in other words, have designed a poisoned chalice for them all.

Do not foretell the future

The European Commission's futures research project has not seen the future; but nobody can.

In Brussels, FAST means "forecasting and assessment in the field of science and technology", and the European Community now has two substantial volumes of a report on the subject. Among the now copious literature of the future, the report stands out for its sanity — the guiding assumption is that there must be a way in which present economic troubles can be cured by intelligent research, development and capital investment. The document is also full of interest — there is an intelligent discussion of the changing pattern of employment under the influence of changing technology and of what the authors of the report repeatedly call "the international division of labour", usually in a European context. Moreover, recommendations as to where, in the whole field, research and development should be encouraged are not pulled like rabbits out of a hat but are, rather, preceded by a careful account of the present problems of European industries, from chemicals to construction. For all these virtues, however, the report is too disjointed to be persuasive. Worse still, it is unlikely to be the kind of touchstone for planning the future development of the European Community's research programme that the Commission hoped for when the work was set in hand three years ago.

The reasons for this disappointment are easily found, and are probably inescapable in any attempt such as this to foretell the future. First, the forecast provided, necessarily a projection of the present into future decades, is innocent of the surprises yet to come. In this spirit, the FAST report pays particular attention to two now fashionable fields in which technology is likely to have a profound effect on the character of society — information technology and biotechnology. Thus the report has much in common with other recent attempts to plan ahead in research and development, the attempt by a committee of the British Department of Industry to map out a programme of research and development in information technology, for example. Thus, while some seers have for years been prophesying that biotechnology will be important, it is less than ten years since the prospect became as substantial as it is now. Nobody can be blamed, but even the specific account of the future now offered must overlook the technologies that have not yet emerged. A further snag is that committees are now well placed to plan the future strategy of research. Yet while committees have been saying for the past thirty years that the closing decades of this century would be dominated by the technology of telecommunications and computers, it has never previously been possible to spell out just how a government or a corporation should seek to profit from the likely course of development.

For such reasons, it is to be hoped that the European Community as a whole will take most to heart those features of the report now published that deal with structural questions, not the details of which research and development projects are likely to be most valuable in the years ahead. First, there is an urgent need for more incentives, within the Community, for a rational division of labour that will allow the most efficient producers to produce most. Although there has recently been some progress in throwing public purchasing open to all European companies, governments are still able to drag their feet in the interests of chauvinism — and as a result undermine their own long-term prosperity. Similarly, there is an urgent need somehow to make the European system of higher education and technical training more flexible, and to encourage mobility within it. Here again, chauvinism, if not outright xenophobia, is still all too common within Europe. But would it not make great sense that Europe should, for example, work towards some common university system? On both counts, alas, the obvious objection is that if some nations gained from a more efficient division of labour, others would lose. The answer is that this is not a "zero-sum game" but one in which the wealth of Europe as a whole would be improved.

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Further snag with Stanford patent

Filing error claimed in Cohen–Boyer

Washington

The second Cohen–Boyer patent covering basic processes in genetic engineering may have suffered another setback last week when attorneys for the applicants admitted that there is a serious error in the patent specifications.

The error was one of several potential flaws cited by the US Patent and Trademark Office when, in August, it suddenly reversed its previous decision and decided to reject the patent (see *Nature* 12 August, p.595). Responding just one day before a deadline of 2 November, Stanford University and the University of California concede the error, but argue that the patent is nonetheless valid.

Their response, however, may raise more questions than it answers. Attorney Bertram Rowland, representing the universities, does not explain why he failed to inform the patent office of the error, which, he admits, "was known to the applicants' attorney as early as December 1977". And the two lines of defence he introduces in support of the patent's validity may well be disputed.

Rowland argues that despite the error, the patent office should withdraw its objections and grant the patent, and he offers two lines of defence for that argument. The first is that even "if applicants had not actually performed any of the experiments described in the experimental section . . . the specification would be enabling (sufficient to allow duplication and thus be patentable), since it teaches how to obtain and clone a functional plasmid containing foreign DNA". In other words, pSC101 is not itself vital, as the instructions provided in the patent explain how to select, isolate and use other suitable plasmids. "Testimonials" from molecular biologists Donald Helinski, Stanley Falkow and Bernard Weisblum are included to buttress the claim that anyone with "ordinary skill" in the field could, since 4 November 1974, have used the patent to prepare a functional plasmid — even without the benefit of a correct recipe for pSC101.

Yet in a submission to the patent office on 24 June 1977, Rowland seemed to take the opposite view — that pSC101 was indeed vital to the patent claims, and that this "new plasmid found by the inventors" was what distinguished their claims from potentially competing work by others.

The second line of defence offered by Rowland is that even if pSC101 is the key, the patent does not need to disclose how to make it since pSC101 was publicly available at the filing date. A declaration by Cohen, included in the response, states that he made the plasmid available to scientists, subject to two restrictions: "that it would not be passed on; that it should not be used in experiments believed at the time to have the potential to be dangerous". Cohen asserts that "no-one who requested pSC101 was denied pSC101" and that it was thus "widely available".

One patent attorney familiar with the case said that Cohen's restrictions on distribution of the plasmid may defeat this defence; to be safe, he should have deposited the plasmid in a recognized public repository such as the American Type Culture Collection before filing. This was not done until 25 June 1981, six months after the first patent was granted.

Rowland's admission of the error in the second patent application raises a potentially explosive question: should the first patent, which contains the same flaw, be reissued to correct it? Given the problems with the second patent, this is something Stanford would like to avoid. In an interview last week, Rowland tried to avoid the issue, saying, "Given that 99 per cent or more of the people in the field are aware of the situation [the error in the pSC101 recipe], whether I should have to go to the expense of filing for reissuance is an open question". He added, "I don't

Rowland's concession concerns an article published in November 1977 by co-inventor Stanley Cohen and a co-worker (Cohen, S.N. & Chang, A.C.Y. *J. Bact.* 132, 734; 1977) which contradicts the recipe set out in the patent for making pSC101, the key plasmid used for inserting foreign DNA into *Escherichia coli.* "pSC101 could not be prepared in accordance with the working example", Rowland writes.

The *Journal of Bacteriology* article came to the attention of the patent office only this summer, apparently through an article by an Exxon patent attorney, Albert Halluin. In several footnotes to a chapter he contributed to *The Patenting of Life Forms,* a book published in August by Cold Spring Harbor Laboratory, Halluin cites that article and other potential flaws in the patent. Rowland, after admitting that he knew of the existence of Cohen's article five years ago, writes that "I have no recollection as to why it was not brought to the Examiner's attention in the written record". The patent application was filed on 4 November 1974; it was later split into two parts, and the first patent was granted on 2 December 1980.

UK research councils' budget break

British research councils were told this week how much money they will get next year, but not which council is getting what — surely a formula for a scrap until the detailed allocations are announced in a few weeks' time.

According to Sir Keith Joseph, UK Secretary of State for Education and Science, who made a statement to the House of Commons on Monday, the total to be made available is £509.7 million, a shade above the £507 million requested by the Advisory Board for the Research Councils (ABRC) a few weeks ago (see *Nature* 4 November, p.7) However, the new figure includes a Falklands factor — an extra £4 million for the British Antarctic Survey (BAS), in line with policy announced after the Falklands conflict to increase research in the Antarctic. BAS is a component institute of the Natural Environment Research Council (NERC). NERC would welcome the sum as a gift, a spokesman said this week, but would be worried if it were to be subtracted from its planned budget for other science, or from that of other research councils.

Another unresolved issue is the position of the Agricultural Research Council, which has protested against ABRC's recommendation to hold its budget constant in cash terms for the next three years at £46 million.

As for the Social Science Research Council, "a small portion" of the £6 million cut threatened recently by Sir Keith Joseph will fall this year, with more to come in the next two years. It will contribute to a £14 million fund to provide "the first instalment of a programme for new technologies, including the recruitment of young researchers". Of this, £4 million will go to establish courses in information technology in technical colleges and polytechnics. The remaining £10 million is for new blood as well as for the provision of courses in new technologies "primarily to improve the supply of manpower in information technology". Moreover, part of the sum will be allocated not by the University Grants Committee and the universities, but by the research councils, said Sir Keith. So it is impossible to determine exactly how many jobs this £10 million will make available. A separate announcement will give details.

As for the universities themselves, they will enjoy a small increase in their grant for the present academic year (1982–83) from £1,137 million to £1,150 million, and the recurrent grant for 1983–84 will be set at £1,213 million. These figures are fixed in cash terms, and assume a salary increase of only 3.5 per cent. **Robert Walgate**

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know any law that says I have to''.

One old question that has resurfaced in the latest exchange between Stanford and the patent office, and that may bear on the first patent, is whether Cohen and Boyer are the sole inventors, as Stanford and the University of California claim. In its notice of rejection on 2 August, the patent office cites a statement by Dr Robert Helling — a co-author of the 1973 paper in the *Proceedings of the National Academy of Sciences* that is the basis of the patents that appeared in a news story in the 3 April 1980 issue of *Nature* (p.388). Helling, now at the University of Michigan, is quoted as refusing to sign a disclaimer of inventorship as requested by Rowland.

Helling has not, however, pressed any claims to date. And Rowland, in his response to the patent office, asserts that Cohen and Boyer are the sole inventors, having conceived the idea at ''a now famous delicatessen in Hawaii'' during a scientific meeting in November 1972. This, Rowland writes, rebuts ''any implication that the refusal to sign a disclaimer might be equated with an allegation of co-inventorship''.

But Helling told me last week ''I certainly am a co-inventor. The three of us were equal partners. I went out there to develop new cloning procedures; Herb (Boyer) was the one who suggested using plasmids, and asked me if Stan (Cohen) could join us''. Helling worked with Boyer at the University of California at San Francisco during his 1972-73 sabbatical.

Helling has been discussing the matter with the University of Michigan's attorney and said ''we may do something now'', but declined to give any details. A call to the attorney was not returned.

Although a recent court decision overturns the patent office's policy of considering co-authorship *prima facie* evidence of co-inventorship, Helling's statement in the 1980 *Nature* story complicates the matter.

Rene Tegtmeyer, the assistant commissioner for patents, said his office will act ''promptly'' on the case, perhaps reaching a decision within ''two or four weeks''. If the patent office stands by its rejection on 2 August, Stanford will then have three months to file a request for reconsideration or a notice of appeal to the Patent Office Board of Appeals. A decision might not be reached before the summer of 1984 — and the case could go on still longer if the board's decision were to be referred to the Court of Appeals for the Federal Circuit.

Stephen Budiansky

Science and Engineering Research Council

Big money for big science?

A potentially important difference of emphasis between the British Science and Engineering Research Council (SERC) and the Advisory Board for the Research Councils (ABRC) was apparent at the publication of the research council's report (HMSO, £4) last week. For while the advisory board said in its advice to the Secretary of State for Education and Science that the time had come to halt the retreat from Big Science (see *Nature* 4 November, p.1) SERC chairman Professor John Kingsman refers to ''broadening out from the support of 'big science' to a better spread of science and engineering''. Kingsman says that the advisory board's recommendation would be only one element in the council's decision how best to spend its money next year.

SERC's principal area of concern is the dual-support system through which funds for science research are provided jointly through the University Grants Committee (UGC) and the research councils. The SERC report says little about how far SERC is prepared to go to compensate for the dwindling UGC contribution, but also claims that ''the quantity and quality of research grant applications are being maintained''.

Professor I. Butterworth, chairman of SERC's Nuclear Physics Board, is more pointed, saying that the board wants SERC to provide a certain number of permanent posts in nuclear physics attached to universities until UGC is once again able to play its part.

Since 1978, the proportion of support for nuclear physics within SERC's overall budget has dropped from more than 30 per cent to about 20 per cent. The intended opening at Daresbury of the Nuclear Structure Facility (a heavy-ion accelerator) after an extended delay will provide some stimulus but, according to Professor Butterworth, its full utilization will be delayed. There is also a fear that if full support is given to LEP, the electron-positron collider at CERN in Geneva, it may not be possible to fund other particle physics experiments.

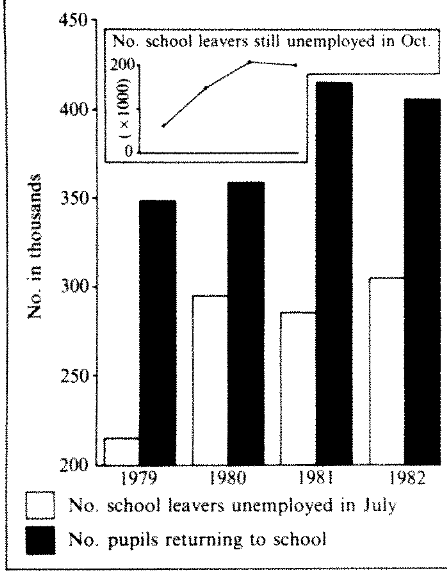
To judge from the report, physicists are also concerned that support for such fields as atomic spectroscopy, semiconductor research, superfluidity and superconductivity appeared to be decreasing relative to such fields as biology, chemistry and applied mathematics. Apparently a special committee has been considering criteria to be used in deciding the relative balance of support between different fields, but its conclusions have not been made public. And there is a possibility that the Synchrotron Radiation Source at Daresbury, from which X-ray spectroscopists have derived particular benefit, may be affected by rising costs, which may make necessary a choice by the Science Board between research grants on the one hand and further development and general support of central facilities on the other.

Support for information technology and space science, both recommended by the Advisory Board for the Research Councils, are, however, both booming at SERC. The two fields are beginning to link up, with the recent development of remote control of telescopes via satellite links between the Royal Greenwich Observatory and telescopes in Hawaii. Such developments might pay for themselves in reduced travel budgets for astronomers.

On the more contentious issue of the use of the South African Observatory (see *Nature* 23 September, p.291), the council says it is not in the business of taking political decisions at the expense of the interests of science and that, moreover, it had felt little pressure from the community and none from the government to sever its links with its South African equivalent.

The press conference called by SERC was made all the more bland by the decision not to make the report available in advance. Thus SERC, proud of its autonomy, has followed a government directive resulting from the premature publication in the national press of details of medals awarded for the Falklands fighting.

Philip Campbell



As the probability of long-term unemployment for the British school leaver has increased (reflected in the October unemployment figures), the number of pupils returning for further education has gone up. There has been no increase in resources made available to schools and extra pupils are being swallowed up by existing classes. However, fears that this would lower the standard at GCE 'O' and 'A' level are not borne out by statistics published by the examining boards, which show no real change in the pass rate during this period. The decline in numbers returning to school in 1982 is at least partly due to the rapid expansion of the Youth Opportunities Programme during 1981-82 from 360,000 to 553,000.

Melanie Kee

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US elections

Key posts held

Washington

Last week's congressional elections, which gave the Democrats a 26-seat gain in the House of Representatives, returned all but two of the senators and congressmen who hold key positions on science and environment committees. The two losers were Senator Howard Cannon (Democrat, Nevada), the ranking Democrat on the Commerce, Science and Transportation Committee, and Senator Harrison Schmitt (Republican, New Mexico), a former astronaut and the only PhD scientist running for the Senate.

Cannon, who lost in a close race in his increasingly Republican state, has been a senator for 24 years. He was recently instrumental in pushing through legislation to guarantee continued funding of the Landsat remote-sensing programme.

Schmitt, who is completing his first term, has been a staunch supporter of President Reagan's economic policies, although he recently went against the Administration in sponsoring legislation to increase spending on science education. Schmitt also supported a shift in funding from the Department of Defense to the National Aeronautics and Space Administration for shuttle operations and for increased space and aeronautics research.

Three supporters of environmental legislation in the Senate who faced strong opposition in the election will all be back: Robert Stafford (Republican, Vermont), George Mitchell (Democrat, Maine) and John Chafee (Republican, Rhode Island).

In the House of Representatives, all the key players on science and the environment committees will be returning, although George Brown (Democrat, California), a strong advocate of science education and research, was pushed to sending out a plea during the campaign for financial support from scientists and educators.

At the other extreme, Senator William Proxmire (Democrat, Wisconsin) of Golden Fleece Award fame proved much more popular among his constituents than among the nation's scientists. He wound up with 64 per cent of the vote, having spent less than $150 on his re-election campaign.

A resolution calling for immediate negotiations toward a bilateral nuclear freeze was passed in eight states. Although the resolutions are not binding, the Reagan Administration had campaigned heavily for their defeat, arguing that a freeze would endanger US security. Voters in Massachusetts, Michigan, Montana, New Jersey, North Dakota, Oregon and Rhode Island passed the measure by a wide margin. It narrowly passed in California and failed in Arizona, the only other state where it was on the ballot paper.

Stephen Budiansky

West Germany science

Federal plans

The implications for science of the change in government in West Germany are emerging only gradually. The new CDU/CSU/FDP coalition is clearly going to be cautious in what it does at least until after the federal election next March. In any case, the vast and decentralized structure of German science makes it impossible to implement changes quickly. An indication of the new team's plans has been given by Dr Heinz Riesenhuber, CDU minister at the Research and Technology Ministry (BMFT) in speeches at a joint industry/BMFT seminar on technology transfer and at a meeting of the Fraunhofer-Gesellschaft in Munich.

Dr Riesenhuber made it clear that direct support of individual projects will be run down in favour of indirect support through tax relief, low interest loans, allowances and subsidies. The rundown can be seen as a continuation of the previous administration's policy under which barely half as many projects were started in 1981 as in 1979. Dr Riesenhuber hopes that indirect support will encourage more market-oriented research and he singled out the energy saving programme for particular criticism, saying that the returns for the DM 4,350 million (US $1,700 million) spent are quite inadequate.

In a similar vein, at the technology transfer meeting, the minister demanded that work at the 12 large state-supported research centres should concentrate more on market-oriented work and suggested that there should be more independent co-operation between research and industry. He expressed a belief that there is a pool of research results which could lead to industrial innovation at little or no cost. This seems questionable, for while it may have been possible to carry out development on a shoestring fifty years ago, modern requirements for quality and reliability in products and processes have made the step from research to production both expensive and time-consuming. If Reisenhuber's pronouncements are a foretaste of the kind of shift in emphasis that has occurred in science budgets in the United Kingdom and in the United States during the past decade, scientists working in basic research and on long-term projects will have to fight hard for their corners.

In more concrete terms, the draft budget for 1983 shows that the immediate effects will be small. The total research budget is DM 6,911.8 million (US $2,710 million), a decrease of 2.4 per cent compared with the draft budget of the previous government and of about 2.2 per cent compared with 1982. Details have not yet been announced and the proposals may be modified when they are discussed in the Bundestag on 11 November.

J.S. Dunnett

Interferon therapy

Side effect scare hits French trials

Paris

Four patients out of eleven being treated for cancer with French alpha-interferon have died from the same cause this year in France — myocardial infarction, a failure of a section of heart muscle through the loss of local blood supply. As a result, Jack Ralite, the French minister of health, has cancelled the trials, at least until Institut Pasteur Production (IPP), which produces the drug, can repurify its stocks and perform new toxicity tests on the material.

The deaths could be attributable to the interferon itself, or to impurities in it, or it could be just a run of bad luck. Several other countries are performing human trials with interferon and there have been no reports of the heart disease syndrome, and IPP uses essentially the same production method as Dr Kari Cantell, who has supplied much of the world's alpha-interferon: extraction from blood-bank leukocytes.

But the sequence of four similar deaths was too much to ignore. The scientific council which was set up to advise Ralite specifically on trials with the IPP interferon (the only French source) advised him to halt the trials. According to the president of the council, medical statistician Professor Robert Flamant, suspicions were first raised when the first two patients both died of myocardial infarction near the end of their treatment. They were both women 50–60 years old with acute breast cancer, who had been treated by other drugs and with radiation. The deaths led to "discussions" in the council, but they were dismissed as coincidence.

There was no problem with the third patient treated — nor any cure. But the fourth, a kidney cancer case, died of myocardial infarction actually during one of the interferon infusions.

At this point the council decided to change the method of introduction of the drug, which until then had been the "more aggressive" intravenous route rather than the intramuscular route which is usually preferred but which limits the size of the doses. This was fine for six patients, but in the seventh, with a slight increase of dose, there was another myocardial infarction. It was "less clear" in this case that interferon might be to blame, as the patient had a history of slight heart trouble. But it was enough to lead the council to stop the tests, especially since they also noted that a child had suffered great shock — and just survived — when given an injection of the interferon for a virus infection.

Flamant, however, expects his council to reauthorize the trials when IPP has repurified its stock (leukocyte interferon is always contaminated with various other proteins besides interferon) and if the toxicity trials — to be performed on dogs — are completed successfully. This may take two months, he estimates.

There is anxiety, however, about non-French interferon, over which Flamant's council and the minister himself have little or no control. The council deals specifically and solely with IPP interferon because it arose as a *quid pro quo* when the government first offered a guaranteed market to IPP for its product. Having guaranteed the cash, the government also wanted to ensure proper statistical control of the trials and so established the council.

But non-French interferon enters the country with no more than an import licence — and some of it, it is suspected, without any licence at all. This foreign interferon is being used for phase 1 and phase 2 human trials without any control, and with little knowledge on the part of the ministry.

It is therefore being suggested that the foreign interferon material, and other experimental drugs as well, should be controlled under a comprehensive oversight scheme. Thus questions raised over the purity of a particular kind of French interferon may also affect other drugs, both French and foreign.

Robert Walgate

Anglo-Australian bomb tests

Government traces victims

Canberra

It seems that the dust has not settled over Maralinga or at a site 200 kilometres to the north of it called Emu, both in South Australia, nor over the Monte Bello Islands off the coast of Western Australia. It was in these remote spots that the British government conducted a series of 12 atom bomb tests between 1952 and 1957 (see table). In addition, in the following six years, weapon experiments involving radioactive fallout, though not from explosions, were undertaken at the testing range of Maralinga — an aboriginal word meaning "field of thunder".

The federal government is now trying to trace people associated with the tests, some 15,000 in all, in order to establish if there might be a link between mortality and morbidity of this group and the tests. On 22 October, the Minister for National Development and Energy, Senator Sir John Carrick, announced that questionnaires seeking medical data were sent to all the 8,000 people whose whereabouts are known through sifting administrative records, and he appealed for help to find the rest. The Department of National Development and Energy, which is conducting the survey, is also unearthing death certificates for information on the number and causes of death among this population. Furthermore, the government commissioned an independent body of experts, the Australian Ionising Radiation Advisory Council (AIRAC), to examine the adequacy of safety precautions at the time and the possibility of ill effects from fallout. This report, now in its final draft, was expected to be released in a month but it will now be delayed until next year, partly because it contains some classified information awaiting clearance by British authorities.

The survey is both a response to and an attempt to allay growing public disquiet in the past three years over newspaper reports, mainly from South Australia, of illness and death among servicemen involved in the tests. Another story, just as alarming, which may not be apocryphal, is about a tribe of the Pitjantjatara aboriginals wandering into ground zeros during the tests unbeknown to the British. An attempt by the South Australian Health Commission to authenticate that claim only served to highlight the difficulties of obtaining information from a small nomadic and dispersed group and the lack of control data for comparison.

Of the many compensation claims lodged at the office of the commissioner for employee compensation, liability has been acknowledged in 5 cases — 3 for deaths from cancer, 1 for the aggravation of an existing nervous complaint, and 1 for a thyroid disease. The largest sum, A$32,000, was paid to the widow of Mr Frank Eaglen who died of cancer. Claimants are aided by the fact that the onus of proof rests with the government.

Australia and the United Kingdom agreed to establish a testing range at Maralinga in August 1954 and it was approved by cabinet in May 1955. Subsequently the Atomic Weapons Safety Committee was instituted, whose job it was to ensure that the tests were conducted to the satisfaction of the Australian government. This committee of scientists had the power to veto, until the moment of firing, any test not meeting its safety criteria. After its closure, the range was partially decontaminated in 1964 and again in 1967 (operation "Brumby") by British teams. However, as a consequence of Australia's ratification of the nuclear non-proliferation treaty, Britain was asked to "repatriate" half a kilogramme of plutonium existing as a single discrete mass buried at Maralinga and the material was removed in 1979, the same year in which the Department of National Development and Energy completed a programme of waste management and rehabilitation in accordance with AIRAC recommendations.

Vimala Sarma

UK atomic tests in Australia 1952–57

Code	Location	Firing site	Date	Size
Hurricane	Monte Bello	Off Trimouille Island	3 Oct 1952	Kilotonne
Totem 1	Emu	—	15 Oct 1953	Kilotonne
Totem 11	Emu	—	27 Oct 1953	Kilotonne
Mosaic G1	Monte Bello	Trimouille Island	16 May 1956	Kilotonne
Mosaic G2	Monte Bello	Alpha Island	19 June 1956	Kilotonne
Buffalo	Maralinga	One Tree	27 Sept 1956	Kilotonne
Buffalo	Maralinga	Marcoo	4 Oct 1956	Low yield
Buffalo	Maralinga	Kite	11 Oct 1956	Low yield
Buffalo	Maralinga	Breakaway	22 Oct 1956	Kilotonne
Antler	Maralinga	Tadje	14 Sept 1957	Low yield
Antler	Maralinga	Biak	25 Sept 1957	Kilotonne
Antler	Maralinga	Taranaki	9 Oct 1957	Kilotonne

Source: Australian Ionising Radiation Advisory Council Reports Nos 4, 5 and 7 tabled in the House of Representatives on 30 May 1979, 13 November 1979 and 22 May 1980 respectively.

Radiation exposure

Unions agree

A scheme for compensating the dependants of radiation workers who have died from cancer has been agreed between British Nuclear Fuels Ltd (BNFL) and its trade and staff unions. The scheme will apply to present and past employees, but requires that dependants will be eligible for compensation only if there is evidence that the cancer may have been caused by occupational radiation exposure.

British Nuclear Fuels is the publicly owned monopoly for reprocessing and fabricating nuclear fuel. The distinctive feature of the new agreement is that compensation will be determined by the probability that a cancer has been induced by radiation, thus avoiding the "all or nothing" conundrum that has complicated earlier legal cases.

The scheme is hailed as a "pioneering deal" by Mr John Edmonds, National Energy Officer of the General and Municipal Workers Union (the main union involved in the negotiations). It is certainly a unique scheme in the nuclear industry, although large employers in other industries run compensation schemes that work along similar lines. Previous claims in respect of radiation-induced disease made through the courts have resulted in very long delays in payments; several substantial out of court settlements have been made.

The new procedure is voluntary, but the unions involved will recommend claimants to make use of the scheme. The option of taking a case to court instead is not

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excluded, and a compensation payment under the new procedure does not constitute an admission of liability by BNFL.

BNFL is to examine radiation dosage records of its employees at Sellafield (formerly Windscale) and its other plants, and will include employees of its predecessors in the field, the Atomic Energy Authority and the Ministry of Supply, going back to 1950. A BNFL spokesman, while emphasizing the company's continuing efforts to minimize radiation exposure among its employees, estimated that between 10 and 40 additional payments are expected immediately under the new scheme.

In difficult cases an independent panel of experts, jointly appointed by BNFL and the unions and under the chairmanship of Lord Gregson, will decide the level of compensation. The agreement is provisionally scheduled to last for two years and will be extended if it proves successful. It is also hoped that it might be possible to extend the scheme to cover cases in which radioactive material has been ingested (more difficult to assess for technical reasons) and cases of disablement. **Tim Beardsley**

EEC research council

British block safety reactor

Brussels

An otherwise successful meeting of the EEC's research ministers in Brussels last week was marred by some tough British objections to the new budget put forward by the Commission for the Super-Sara project. The project, which aims to explore ways of managing crises in light water reactors when loss of coolant takes place, is included in the new four-year (1982–86) programme for the EEC's joint research centres (JRC). Super-Sara will absorb a considerable part of JRC's budget at a time when efforts are being made to restructure its work so that it is more in line with other research and development aims of the Community.

Super-Sara was first proposed after the Three Mile Island accident and when the Italians wished to find a use for the Essor experimental reactor which was no longer being used at the Ispra Research Centre. Since then the enthusiasm of the rest of the member states, never very strong, has waned considerably as the original cost estimates have been continually revised upwards. British officials were predicting before Thursday's council that the project could be scrapped and even now its future is by no means certain.

A decision will have to be taken by Coreper, the committee of the member states' ambassadors, within the next two weeks before the revision of the 1982 budget is completed. Mr David Mellors, British Under-Secretary of State for the Department of Energy, has now asked the European Commission to present further details on how the increased project costs can be accommodated and has questioned whether the project, which he thought had been badly managed until now, could still be considered cost-effective.

Mellors said that the results that the project would yield could not be considered essential since it investigated only an extremely unlikely type of malfunction. The Commission has, however, received support from a task force of experts whose report concluded that the project was worthwhile and fitted into the matrix of research being carried out elsewhere. And even the United States has until now shown interest in contributing to the cost of the project. British opposition might be rooted in national interests because, having chosen to build pressurized water reactors, the United Kingdom will be only marginally interested in the results of the project.

Considerable confusion however, surrounds, the various estimates of Super-Sara's cost. An early estimate of 139.53 million European currency units to cover the project until 1988 has been constantly revised, standing now at 293.9 million units (£161.7 million).

European Commissioner Viscount Etienne Davignon told the ministers that 10 million ECU and 17.5 million ECU urgently needed to be taken from the 1982 revised and 1983 budgets respectively, plus a further 15 million ECU each for 1984, 1985 and 1986, if the project was to continue at all. He also pointed out that more delays will increase the cost of the project still further and that the cost of closing down the Essor reactor would not be cheap either.

Part of the explanation for the dramatic rise in cost estimates lies with the nature of the revision of JRC's programmes. Next year will be a special bridging year between the last programme (1980–83) and the 1984–87 programme. More staff are being recruited and 161 new posts are being created, 81 of them for the Super-Sara project, although the Commission claims that this is a temporary expansion to cope with the expected retirement of senior scientists. It now seems likely that instead of new staff being engaged there will have to be redeployment of staff among JRCs and national laboratories or certain other projects or plans will have to be curtailed such as the increase in JRC research asked for in nuclear energy. Except for the possibility of the number of experiments being reduced, there appears to be very little room for compromise over Super-Sara itself, and the British are worried about putting too many eggs in one basket. Even under present calculations, the fast breeder safety and the fuel cycle safety programmes are being cut back.

Jasper Becker

Thermonuclear fusion

Padua approved

Brussels

It is now virtually certain that the reversed field experiment (RFX) which was originally to be funded by the UK Atomic Energy Authority at the fusion research centre at Culham, will be taken on by the Italian Comitato Nazionale Delle Richerche and built at its laboratories in Padua. The consultative committee of the fusion programme was given the go-ahead to both this project and the construction of the Wendelstein VII advanced stellarator project, to be built at the Garching Laboratory of the Institut für Plasmaphysic in Germany.

Researchers were disappointed last year when the British government decided that there was no room in Culham's budget for the British contribution to the experiment of 16.5 million European currency units (ECU, 1 ECU = 55p). RFX is a direct descendant of ZETA — the zero energy thermonuclear apparatus — which hit the headlines in the late 1950s when it failed to achieve fusion for reasons which were not understood for some years. G.B. Taylor in the next decade proposed the reverse field theory to explain the failure and it was hoped that Britain could continue to lead the world in this field.

Reversed field pinch is one of the three options for achieving fusion which are being pursued in Europe under the Community's research programme. The experiment which will now be started in Padua will involve the construction of a 2,000 kA machine which will enable the experimenters to achieve a longer plasma confinement time. Padua had in 1978 been successful with a much smaller machine, known as ETA-BETA, capable of producing plasma currents up to 250 kA.

The project will still involve staff from Culham but the Italians will be providing the bulk of the cost of the experiment, some 15.7 million ECU, which will start next year and cover about four and a half years. The Community's budget will meet the rest of the cost, totalling 28.5 million ECU although this may rise, depending on whether the US Department of Energy is still willing to contribute to the cost of the project.

Officials in Brussels are, however, confident that the project may now go ahead without further setbacks. In fact, there may be advantages in siting the experiment in Padua where it will not have to play second string to the JET tokamak.

The Wendelstein VII advanced stellarator project will be the first stellarator to use modular twisted coils instead of helical windings to create the poloidal half of the magnetic field that heats and compresses the plasma. The stellarator will cost 12 million ECU to build and the Community will be contributing 5.4 million ECU.

Jasper Becker

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NSF gets new director

Almost as soon as John B. Slaughter, the outgoing director of the US National Science Foundation (NSF), the nation's basic research agency, announced that he would leave on 1 November to take up his duties at the University of Maryland, the White House announced his successor. On 3 November Edward A. Knapp assumed his new duties as director.

Knapp, a physicist with degrees from Pomona College and the University of California at Berkeley, had been confirmed in September as assistant director of NSF for mathematical and physical sciences. His entire previous career was with the Los Alamos National Laboratory, where he worked with George A. Keyworth, who is now the President's Science Advisor. Ironically, the White House personnel office made the appointment with despatch while Keyworth, Slaughter and NSF's deputy director Donald Langenberg — who was rumoured as a likely candidate — were out of town.

Knapp's work at Los Alamos began in 1958. He worked on controlled thermonuclear research and helped to plan the linear proton accelerator, the Los Alamos Meson Physics Facility (LAMPF). Knapp spent a year at CERN in Geneva, where he worked out a collaborative programme between CERN and Los Alamos. Before coming to Washington Knapp was director of the laboratory's accelerator technology division.

Since Congress is in recess at present, Knapp is assuming his duties without being formally confirmed. Langenberg plans to stay on, although his name is on a list of candidates for the presidency of the University of New Mexico. At the time that Knapp was appointed, there were rumours in Washington of a major conservative housecleaning at NSF, which could not be confirmed. Moreover, the national elections, which seemed to deprive the Reagan Administration of some of its far-right conservative mandate, make such a sweep very unlikely. **Deborah Shapley**

French energy

Forecasts fall

In the midst of an appalling economic crisis, the French government is about to be struck another blow with the expected publication this week of an official energy forecast which will lower projected electricity consumption in 1990 by nearly a quarter.

The consequences fall neatly on the coal and nuclear industries, both of them political hot potatoes for a socialist government. Coal and nuclear energy are the dominant power sources in France (producing 24 per cent and 37 per cent of delivered electric power in 1981), excepting hydroelectricity (27 per cent) which, as nearly "free" power, is likely to be untouched by the new forecast. The socialist party has committed itself to support the coal industry — and indeed expand production — but the nuclear lobby is strong and supported vociferously by the powerful research and industry minister, Jean-Pierre Chevènement.

The new figures come from the Commission du Plan, an institution which is both within and to some degree aside from the government. But its pronouncements — broad political and economic plans for the future — are taken seriously. In this case, the "long-term energy" committee of the commission has calculated that electricity consumption will not be 450,000 million kWh in 1990 as envisaged in the previous (1980) plan, but only 350,000 million kWh, compared with a true consumption in 1980 of 246,000 million kWh. Thus the projected increase has been halved, with a consequent revolution in thinking about power station construction and power sales.

Electricité de France (EDF), which is strongly behind nuclear power, contests the figures and predicts that consumption will be 390,000 million kWh in 1990; but it still admits that there will be overcapacity. EDF argues that surplus electricity can be exported — French electricity is the cheapest in Europe, largely because of government pricing policy — and that the nuclear power industry must build at least two stations a year until 1990 or it will collapse. A successful nuclear industry will lead to nuclear exports later, the argument goes. But if nuclear power station construction continued at the present rate of four stations a year, it is estimated that coal burning for electricity — which makes up half the French coal market — would fall from its present level of 64,000 million kWh to 32,000 million kWh in 1985 and even less in 1990.

With little union backing for coal (the communist CGT union, which is strong in both the coal and nuclear industry has opted for nuclear energy), and with Chevènement behind nuclear power, it seems likely that the EDF view will be supported, and a substantial nuclear programme maintained, with unpleasant consequences for the socialist party in the French coal districts in the municipal elections in the spring.

Why then are the forecasts falling? The answer is twofold. First, French economic growth is well below target, and seems unlikely to rise before 1985; and second there is growing confidence in the Agency Française pour la Maitrise de l'Energie (AFME), which seems to have had considerable success since its formation earlier this year from a group of disparate bodies concerned with energy conservation and renewable energies.

In 1983, AFME will invest FF1,200 million (£100 million), much of it in investment support grants which will raise a total of FF20,000 million (£1,300 million) in conservation and alternatives next year. The aim is to reach FF40,000 million (£2,600 million) a year by 1990, comparable with the present French investment rate in nuclear power. Some of the seed money is being raised by a tax on petrol at the pump.

By 1990, AFME is expected to have led to renewables usage of some 12 million tonnes of oil equivalent a year (around 10 per cent of primary energy consumption) with the greatest portion coming from wood and biomass, then from low-temperature geothermal heat (particularly by better exploitation of sources in the Paris basin) and finally from solar power. But the great saving comes in energy conservation — around 40 million tonnes of oil equivalent a year by 1990, according to AFME projections, which are broadly accepted by the Commission du Plan.

Robert Walgate

Genetic engineering

Human insulin

Washington

The commercial potential of genetic engineering was boosted in the United States on 29 October when the Food and Drug Administration (FDA) gave marketing approval to Humulin, a human insulin made with genetic engineering techniques. Made by Eli Lilly Company, the substance had won approval for marketing in the United Kingdom only a month before (see *Nature* 23 September, p.293). Although the announcement has symbolic value in the field and in particular for Genentech Inc., the California company that invented it, it will probably not quickly make a material difference to the fortunes of either Genentech or Eli Lilly.

Genetically-engineered human insulin is expected to be almost twice as expensive as the insulins now derived from pigs and cattle, which cost an estimated 26-30 cents per day of treatment. Lilly already has an estimated 85 per cent of the insulin market in the United States, so there is not likely to be a large market for Humulin soon, although the new product has an advantage in the few diabetics who develop an allergy

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to animal insulins.

The announcement of FDA approval had been so long expected that neither Genentech's nor Lilly's stock jumped much in response, although Lilly's stock, which has been depressed since its sudden withdrawal of the anti-arthritis drug Oraflex earlier this year, traded a little higher after the FDA announcement on Humulin.

FDA gave approval on the basis of safe performance in trials involving about 400 patients a mere five months after Lilly filed its application. The agency also has in the pipeline applications for genetically-engineered products such as human growth hormones and interferons. Approval can take as long as two years, but the agency has recently undertaken a series of controversial moves to shorten approval time and simplify procedures.

Genentech is the first of the highly competitive biotechnology companies with a product approved for human use. The company's task was enormously eased by the security of the support provided by Lilly, which has exclusive licensing rights and which is estimated to have spent $100 million in bringing the product to market. Genentech will receive a royalty on sales which is thought to be in single figures.

Genentech may be following a riskier path in its development of human growth hormone products and gamma interferon. It plans to market these products itself, which explains why it persuaded a group of investors to buy limited partnerships in October, thereby raising $55 million for research. This will support work on a human growth hormone product for children who suffer from dwarfism, but since these are few in number the product would also have to be useful in combating CDSS (constitutionally delayed short stature), a problem that afflicts a greater number of people. Ideally, such a product could also help patients with cachexia, a wasting syndrome.

In addition to the limited partnership, in October four Swedish organizations bought $20 million in Genentech's outstanding shares, or 571,428 shares at $35 per share. (Two weeks later, after the limited partnerships and the FDA announcement, the company's stock reached $50 a share.) The four organizations are Alfa-Laval AB, the Wallenberg Foundation, AB Fannyudde, an investment company affiliated with Volvo, and D. Carengie and Co., another investment company. The president and chief executive officer of Alfa-Laval AB, Harry Faulkner, was made a member of Genentech's board of directors. The company also appointed to its board John T. Potts, chief of the Department of Medicine at the Massachusetts General Hospital. Dr David W. Martin, a professor of medicine and biochemistry at the University of California, San Francisco, has been appointed vice-president for research.

Deborah Shapley

Biotechnology index

Biotechnology index beats Dow

Washington

Nature's third monthly Biotechnology Index set a new high in October, reaching 140.2 from a base of 100 at the end of June. This increase was greater than either the recent increases in the Dow Jones Industrial Index, the leading index of US industrial trading, or the Standard & Poor's 400 index of leading industrial stocks. Thus, the biotechnology companies moved parallel to, but ahead of, the market surge that led the Dow Jones index finally to break through the 1,000 mark in the first week of October.

But the performance of the *Nature* Biotechnology Index may have as much to do with internal factors in the companies themselves as with trends on Wall Street. The "Street" was reacting to continued lowering of inflation and the perception that the US elections, just held, would not upset the direction of the US economy. As one analyst in New York explained, the overall market surge was caused by "institutional money managers getting in who don't want to miss more of the rally". Such managers buy huge blocks of stock and so stay away from smaller companies where such buying would artificially drive up the price.

The analyst went on to suggest that one reason why the *Nature* index did so well was because two companies in the index are large enough to attract some institutional funds and stable enough to ride along with the trend. These are A.B. Fortia of Sweden and Novo Industri A/S of Denmark, which had market capitalizations valued at $751 million and $789 million respectively on 29 October, when the index was calculated. The reasons for the rise in stock price of Genetech are outlined in the preceding story.

The other small companies in the index have also done well lately. "There have been no major flubs in the group", one analyst says. For example, the stock of Cetus, in Berkeley, California, rose after the company announced lay-offs and a re-direction of its research. This was seen as a net gain for the company, because specialists in biotechnology investment had felt that Cetus was over-extended. Hybritech and Damon of Needham have been getting considerable public attention for their work.

Deborah Shapley

Nature **index of biotechnology stocks**

1982 high	1982 low	Company	Close previous month	Close 29 Oct.	Change
45	16⅛	**A.B. Fortia (Sweden)**	34¼	39½*	5¼
8	2	**Bio Logicals (Canada)**	2½	2¼	–¼
7	3⅝	**Bio-Response (USA)**	4¾	5	¼
14⅛	7¼	**Cetus (USA)**	8¾	9¼	1
11	6⅛	**Collaborative Research (USA)**	8⅝	9¾	1⅛
9⅞	5¾	**Damon (USA)**	7½	9⅛	1⅝
18	8¼	**Enzo-Biochem (USA)**	14½	17	2½
28	6⅝	**Flow General (USA)**	10⅞	13¼	2⅜
47¾	26	**Genentech (USA)**	33¼	47¾*	14½
9½	7	**Genex (USA)**	—	9¼	—
5⅛	2¼	**Genetic Systems (USA)**	3¼	5⅛	1
26¼	9⅝	**Hybritech (USA)**	13½	20½	7
11¾	5	**Molecular Genetics (USA)**	10¾	9¾	–1
52	34⅞	**Novo Industri A/S (Den.)**	44	47½	3½
17	8	**Monoclonal Antibodies (USA)**	8¼	14¾	6½

The *Nature* Biotechnology Index for October, 1982, stands at **140.2**, compared with **117.9** last month. Base is 100 as of 25 June 1982. Previous indexes appeared in *Nature* 12 August, p.599; 9 September, p.101; and 14 October, p.573. Close-of-month prices are the closing prices on the last Friday of each month. Where stocks are traded over the counter, the price quoted is the bid price. For stocks traded on the American and New York Stock Exchanges, the price quoted is the transaction price. Data from E.F. Hutton, Inc.

*High or low for this calendar year.

In response to readers' comments on the Biotechnology stock index, its composition has this month been changed. Thus Collagen Inc. — a company that does not use new biotechnology — has been replaced by Genex, an important small company which made its first public stock offering after the close of the previous month's index.

This has been done by deleting Collagen's share of the market value of all the companies on 29 October and reallocating that share proportionally among the remaining 14 companies in the index. Then the total market value of Genex shares on that date (an estimated $106 million) was added to the market values of the 14 companies and a new set of market shares calculated. This new set of divisors will be used in the calculation of future indexes.

In response to a further suggestion, there follows information on the trading symbols and, where appropriate, the stock exchanges on which the shares are traded. Most shares are traded over the counter (through brokers). Fortia (FOPHY); Bio Logicals (BIOLF); Bio-Reponse (BIOR); Cetus (CTUS); Collaborative Research (CRIC); Collagen (CGEN); Damon (DMN — NYSE); Enzo-Biochemi (ENZO); Flow General (FGN); Genentech (GENE); Genetic Systems (GENS); Genex (GNEX); Hybritech (HYBR); Molecular Genetics (MOGN); Novo Industri A/S (NVO — NYSE); Monoclonal Antibodies (MABF).

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CORRESPONDENCE

Ethics of trials

SIR — The article on the proposed trials of the Medical Research Council (MRC) to investigate supplements in the prevention of neural tube defects (NTD) refers to ethical questions (*Nature* 16 September)[1]. The MRC trial protocol conflicts with the revised Helsinki Code adopted by the 29th World Medical Assembly in Tokyo in 1975[2].

Dr Nicholas Wald is quoted as believing that a trial is ethical if "the likely good of the treatment" balances "the possible harm", implying that harm to one set of patients can be offset by benefit of another set, or of society. The revised code says (paragraph I.5): "Concern for the interests of the subject must always prevail over the interests of science and society". Informed consent does not affect this obligation (paragraphs II.6 and II.4).

The judgement in the Nuremberg trial of doctors for unethical human experimentation in 1947 said: "The protagonists of the practice of human experimentation justify their views on the basis that such experiments yield results for the good of society"[3]. This same judgement enunciated the Nuremberg Code which was replaced by the Helsinki Code of the World Medical Association in 1964, revised in 1975.

Paragraph II.3 of the revised code says: "In any medical study every patient — including those of a control group, if any — should be assured of the best proven diagnostic and therapeutic method". The MRC trial is proposing to supplement a random half of patients with folate regardless of whether they are folate deficient, although techniques for assay of folate status are available and folate deficiency can be remedied in most patients.

Paragraph I.1 requires all research involving human subjects to be preceded by "adequately performed laboratory and animal experimentation". Folate deficiency in animals is reported to cause fetal resorption, low birthweight, small litter size, stillbirths and polymorphic malformations involving all body systems, as would be expected from folate's biochemical role in DNA synthesis. Folate deficiency delays development of the central nervous system in young children and is associated with mental illness in adults[4]. Paragraph I.9 requires subjects of human experimentation to be informed of "potential hazards of the study".

Folate is only one of over 30 nutrients known to be essential for normal embryonic growth. The comparative importance of different nutrient deficiencies in increasing the risk of malformations will only be discovered by extensive biochemical measurement of nutrient status. Randomized trials will never produce the answer if only because trials of 30 different nutrients at only two different levels of supplementation would require 3^{30} trials. It can be argued that the MRC trial, because it will give no information on the prevalence of any nutrient deficiency, contravenes paragraph I.4 of the code which requires "the importance of the objective" to be in proportion to the inherent risk to the individual. The £300,000 would be better spent on well-planned biochemical measurements of the tissues or blood of women who have had babies with neural tube defects or some other malformations.

The MRC trial protocol contravenes paragraph I.12 of the code which says:

> "The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with."

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1. *Nature* **299**, 198 (1982).
2. *World med. J.* **24**, 76 (1977).
3. United States V. Karl Brandt in *Experimentation with Human Beings* (ed. Katz, J.) 305-306 (Russell-Sage, New York, 1972).
4. Botez, M.I. & Reynolds, E.H. (eds) *Folic Acid in Neurology, Psychiatry, and Internal Medicine* (Raven, New York, 1979).

CAB taxed

SIR — Many eyes must have been rubbed (and job applications drafted) when they read that employees of the Commonwealth Agricultural Bureaux no longer pay income tax (*Nature*, 14 October, p.572), and less self-interested souls must have wondered about the benefit of this to CAB member governments. The benefit lies in what amounts to a 30 per cent cut in taxable salary for CAB employees. A tax consultant works out what employees would normally pay in tax to the UK government, then hands this amount back to CAB. Take-home pay is thus virtually the same as before CAB's internationalization.

ALLAN ROSTRON

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First to use?

SIR — In the article entitled "Vatican and the bomb" (*Nature* 4 November, p.2) you imply that the group convened by the Pontifical Academy in September called for a "declaration of no first use" of nuclear weapons. This is not true.

An earlier draft of the document, prepared by a group of which I was not a member, did indeed "call upon all nations to pledge never to be the first to use nuclear weapons". Many of those present at the final meeting, including myself, objected strongly to this wording on precisely the grounds you state in your article. The words "to pledge" were therefore omitted from this passage in the final document. This was signed by all the participants, and they included all but one of the fourteen who had prepared the earlier draft.

The final version of the document does not contain any suggestion that nations should make a "declaration of no first use" of nuclear weapons.

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The distinction is important but not, without this explanation, readily apparent.

EDITOR, *Nature*

No to boycott

SIR — In responding to Dr Rowan-Robinson's appeal to end British participation in astronomy in South Africa (*Nature* 14 October, p.574) I must first declare an interest. I was one, if not the first, to propose a unification of observatories in South Africa at a single site at Sutherland and a leading activist in site selection and site testing. I quit British employ in South Africa — with the greatest regret — as Dr Rowan-Robinson would evidently wish others to do, in the expectation that judging by the contemporary official climate I might be deprived of observational opportunities. The establishment of the observatory at Sutherland was a subsequent reversal of policy. Sutherland is a very good site, comparable with those in Chile and superior to those in Australia. Although less well equipped than Siding Spring it offers to the observer relatively long runs at telescopes. Speaking from an experience of 30 years in telescope scheduling, I am certain that these are very often more productive, pound for pound and night for night than short runs on larger telescopes and permit greater flexibility in the choice of programmes. The facilities afforded by the site would be very hard to replace.

As to the question whether British participation condones or encourages apartheid this is more complex than would appear at first sight. Many of the actions of the South African Government are abhorrent to the international community. Boycotts of the type proposed are usually ineffective. Indeed some of the extreme right wing of the Nationalist party might be only too glad to see a diminution of the cultural influence of the English language and traditions. Such a boycott would primarily be a blow to a considerable section of the population who wish to see significant political changes. The South African Government does have a genuine problem of terrorist activity — presumably largely inspired by the Soviet Union which seeks to replace one tyranny by another and to gain control of the crucial oil route round the Cape and the strategic minerals of which southern Africa is the only source available to the free world. It is the hope of all reasonable men that political change will come peacably and that many thousands of innocent and hard working people will not be sacrificed in salving the consciences of those remote from the scene of action.

I believe that the best hope for a peacable solution is to increase rather than decrease cultural links from abroad. In a small way the present influx of scientific visitors promotes this. As to Sutherland itself, it is a dreadful location for the coloured people (there are almost no Bantu in the area) but at the observatory there are six coloured employees with good jobs and good housing. This is not much but it is a positive step towards ameliorating the lot of one section of the population which as far as I could judge from a recent visit is enjoying a period of striking economic advance.

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Anatomy of a human cancer gene

A difference of just one nucleotide distinguishes a human cancer gene from its normal counterpart. All of cancer is not that simple but the molecular biologist's approach should be reinforced.

The pages that follow contain an account of one of the most startling discoveries so far in the long and frustrating search for an understanding of cancer. The discovery is startling because it provides an unexpected answer to a question that has been on many people's minds since March last year[1] and certainly since this June[2,3] — what is the difference between a cancer gene and its benign, physiologically indispensable version to be found in normal cells? This week we publish (on pages 143 and 149) two independent but essentially similar answers to this question from two groups in the United States, Dr Robert Weinberg's group centred on the Massachusetts Institute of Technology and Dr Mariano Barbacid's group at the National Cancer Institute. (Further independent confirmation is already in the publication mill.) And on the two following pages, readers will find a cool but enthusiastic appraisal of the significance of these discoveries from Drs Jonothan Logan and John Cairns, of Harvard University.

The essence of what Weinberg, Barbacid and their colleagues have shown is that in cells derived from a bladder carcinoma, there is a gene which differs from the naturally occurring gene in the simplest possible way — the replacement of one specific nucleotide in the DNA by another. The consequence is that the protein whose assembly is governed by the gene differs from the normally manufactured protein again in the simplest possible way — the twelfth amino acid (measured from the end of the protein molecule carrying a free amino group) is changed; in the bladder carcinoma cells studied, the normal glycine is replaced by valine. Nothing could be simpler.

So why the surprise? The most obvious explanation is that this simple result fits oddly with the opinion that has grown up in the past several years that because most kinds of cancer appear to involve cells that have somehow escaped from the unknown constraints that normally limit the multiplication of differentiated tissues, genetic explanations of cancer would probably turn on some property of the mechanisms by which the activity of various genes is controlled. Indeed, Weinberg and his associates describe how, in their study of what are known as the EJ bladder cancer cells, they first looked for differences from the normal in the region outside the gene proper where controlling elements are usually found. They found none of significance. Their account of how they turned to look for differences within the part of the gene which regulates (through the intermediary of messenger RNA molecules) the assembly of amino acid units into protein molecules is as nice an illustration as there could be of how logic can triumph over incredulity.

Several questions now arise, not the least of which is how such a small difference in the structure of a protein molecule can be responsible for such profound physiological differences in the characteristics of a cell, its conversion from a normal to a malignant cell. The fact that the protein concerned is known only as p21 is to be taken as a sign that little more is known about it than that it has a molecular weight of 21,000. The protein appears to be an enzyme and appears bound to the inner surfaces of plasma membranes (so that the production of an abnormal protein would not be apparent from the outside of a cell even if the abnormality were much more pronounced than that now found). Both the groups now reporting draw attention to the way in which a single amino acid substitution could nevertheless have a profound effect on the activity of an enzyme — Barbacid and his colleagues have used computer techniques to show that the replacement of the small glycine unit by the much larger valine could have the effect of stiffening the end of the molecule, perhaps profoundly changing its catalytic properties in the process. However this may be, one thing is certain — there will now be mounted a determined effort to throw light on the normal function of the p21 protein, and of the other proteins it may influence. That will be a gigantic undertaking.

Even at this stage, however, it is clear that the discovery now reported is not a universal explanation of cancer. Thus Barbacid and his colleagues point to the apparent contradiction between their discovery and the demonstration earlier this year[2] that it is possible to make normal cells cancerous simply by infecting them with DNA corresponding to the normal p21 gene to which has been attached pieces of viral DNA corresponding to those thought to be necessary for the functioning of the tumour viruses known as retroviruses. The result of that procedure is to increase the amounts of p21 produced, which suggests that the amount of the protein generated is also relevant. A little abnormal p21, or a lot of the normal product, seems to have similar consequences for a cell, but for reasons not yet properly understood. And why should there be such a close relationship between the cancer genes now described and the retroviruses? Logan and Cairns point in what follows to the fascinating questions raised by that train of speculation.

So where will it all end? Logan and Cairns rightly point out that the discoveries now reported cannot conceivably amount to an explanation of the general phenomenon of cancer. For decades, it has been all too clear that both the causation and the course of different kinds of cancers are exceedingly varied. The importance of what has now been done is that it points unambiguously to one feature of the biochemical apparatus of the vertebrate cell whose abnormality must be supposed to be critical for the development of malignancy. Inevitably there will now be a hunt for a similar analysis of the other known cancer genes, close on a dozen of which are known.It is in the literal sense marvellous that the techniques of genetic manipulation developed in the past decade should now be used to such good and delicate effect (which is in no sense to belittle the difficulty of what has been accomplished, as a reading of the two articles, each with its interlocking network of individual experiments, will quickly show).

What has happened, as Logan and Cairns point out, is certain to change the way in which cancer research is planned. In recent decades, there has been recurrent tension between those (like Cairns himself[4]) who argue that the prime objective should be to understand the phenomenon of malignancy and those who hold that the problem is so complicated that it would be better to concentrate on identifying the causes of concer — and then to avoid them. Nobody will now pretend that understanding is so close at hand that more empirical ways of avoiding cancer or tackling the consequences can be abandoned, but there is the strongest case for seeing that the huge sums of money available for research on cancer are redirected in the directions to which these new discoveries point, and that they are not exclusively spent by the large laboratories but, rather, by those groups of workers whose flair and versatility seem finally to have put paid to the old slur that molecular biology has done nothing for patients.

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1 Shih, C , Padhy, L.C , Murray, M J & Weinberg, R A , *Nature* **290**, 261-264 (1981)
2 Chang, E H , Furth, M E Scolnick, E M & Lowy, D R *Nature* **297**, 479-483 (1982)
3 Parada, L F , Tabin, C.J , Shih, C & Weinberg, R A *Nature* **297** 474–478 (1982)
4 Cairns, J *Nature* **289**, 353 (1981)

0028-0836/82/450103-02$01 00

The secrets of cancer

from Jonothan Logan and John Cairns

THIS year may one day be seen as the year when a strong sense of order came to cancer research and the long drive to understand the cancer cell really got under way. To appreciate the significance of papers like the ones appearing in this issue (Tabin *et al.* p.143 and Reddy *et al.*, p.149) we have to look back at some recent history.

Molecular genetics has brought to biology a new level of order and understanding. The informational macromolecules have been identified and we can now trace at least some of the simpler programmes in metabolism back to their source in the logical circuitry of DNA. What made this possible, more than anything else, was the discovery that genes could be transferred from one cell to another. Without bacterial transformation and the special transfer of information that occurs during virus infection, it would have been hard to show that genes are made of DNA. Without bacterial mating and transduction, dissection of the operon and its component parts would have been almost impossible. Given the wisdom of hindsight, we can see that even the most detailed catalogue of the changes that occur when *Escherichia coli* is fed lactose could not, by itself, have told us how cells regulate protein synthesis. Fortunately, the constraints imposed by evolution require genes to be capable of functioning in almost any context. In the bacterial cytoplasm, everything can interact with everything else, and trying to analyse it is like trying to understand the economy. In the genome, however, genes battle it out one by one, although they are of course being judged by their effects upon the cytoplasm. It is this wonderful separation of living systems into one arena of great complexity and another of great simplicity that has made it possible to find out how cells know what they ought to be doing.

This message has not yet had its full impact on cancer research. A glance at the current journals will show how many people are still trying to understand the biology of cancer by making inventories of the properties of the cells present at each stage of carcinogenesis. Of course, the transition has been difficult. To anyone accustomed to the delights of bacterial genetics, the vertebrate cell must seem an impenetrable forest, with its vast genome, poorly understood systems for gene regulation and — most important of all — its relative inaccessibility to the techniques of genetics. Nature, however, provided one avenue of approach, the tumour viruses. Despite the slender means at their disposal (a few genes-worth of nucleic acid) they possess the means to make a cancer. This fact alone tells us that the various systems of control in animal cells come together at certain focal points ('nodes' in computer terminology) where they can be subverted by single genes and their unique gene products. As we shall see later, evidence is now emerging that these focal points can be the site of attack for at least two other completely distinct forms of carcinogenesis.

Until recently, the vast size of the mammalian genome effectively prevented any direct search for the cellular genes involved in cancer. Without some selective procedure, searching for a mammalian gene is equivalent to looking, without an index, for an article of this length (2,000 words or 20,000 characters) that is known to have appeared in *Nature* some time since 1900 (that is, somewhere in a text of 3×10^9 characters). The genes were discovered, however, because they confer tumorigenicity when they are picked up by certain retroviruses: the virus was therefore doing the selection and the purification. The genes have come to be called 'oncogenes' as if their primary role were to cause cancer. But it was soon realized that typically they are cellular genes ('proto-oncogenes'), whose function is sufficiently important for them to have been highly conserved over the long period of evolution of the vertebrates. One of them is known to change its level of expression during embryogenesis and so it may be a gene normally concerned with development. Another has been shown to code for a protein kinase capable, in principle at least, of modulating the properties of many different gene products, which is just the sort of operation we might expect to find at a focal point in the regulatory circuits of the cytoplasm. But the function of most of the dozen or so identified oncogenes is not yet known. Presumably, each oncogene is not equally important in every tissue and therefore will be more often involved in some kinds of cancer than in others.

The tumorigenic retroviruses can cause cancer in at least two different ways. The virus can bring in an oncogene which, because it is under the control of the viral promoter of transcription, is inevitably transcribed at too high a level. Alternatively, the virus need not contain an oncogene, but merely provide a strong promoter which, by chance, can then become integrated next to one of the cell's oncogenes; this is a much less efficient form of tumorigenesis and usually succeeds only after a long time interval. To summarize, the product of each cellular proto-oncogene seems to be capable of making a cell into a cancer provided that it is produced to excess or has undergone a change in amino acid sequence.

Because the retroviruses are so adept at picking up, transporting and reintegrating cellular genes, they have drawn attention to the particular genes that can endow them with tumorigenicity. It could therefore have been argued, at least until recently, that none of this has much to do with human cancer which, as far as we know, is seldom brought on by an RNA retrovirus. In the past ten years, however, a completely different way has been found for studying the genes involved in cancer. Certain cell lines (most notably the NIH line of 3T3 mouse fibroblasts) have been found to be capable of taking up raw DNA and to be transformed to permanent tumorigenicity if the incoming DNA has been derived from animal, or human, tumours or tumorigenic cell lines. Since human DNA contains certain telltale sequences, not found in mouse DNA, it has been possible (given the formidable armoury of restriction enzymes and all the other paraphernalia of genetic engineering) to isolate these human genes that are tumorigenic for NIH 3T3 cells and presumably were at least partly responsible for the cancers from which they were derived. The fact that such experiments are possible is, in itself, a dramatic demonstration that alteration of the genome is an essential step in the production of some cancers. At this point, it is simplest to list the other major conclusions to have come from these studies of 'transfection'.

First, it is just possible, with great difficulty, to transform NIH 3T3 cells by feeding them their own DNA, provided that it has been fragmented into roughly gene-size pieces; but these transformed cells are then an efficient source of transforming DNA, provided that it is not highly fragmented. By analogy with the retroviruses, it seems likely that the first, and difficult, step is to bring one of the cell's proto-oncogenes under the control of one of the cell's strong promoters, but once these have been brought together they can easily transform other cells, provided that they are not separated.

Seond, when efficiently transforming DNA arises in naturally occurring tumours, or is found in retroviruses, it can function across species barriers; for example, human tumour DNA can transform mouse cells. This is what we might expect, since the oncogenic sequences have been so closely conserved during evolution.

Third, in several instances, the tumorigenicity of DNA from human tumours has been traced to the activation of one of the oncogenes that had been identified in murine sarcomas (either the Harvey or the Kirsten *ras*), suggesting that a change in the function of either of these can be a step in the formation of cancer in several organs. Interestingly, in the examples studied in this issue of *Nature*, the tumorigenicity of DNA seems to be

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due, not to a change in level of expression of the oncogene, but to a change in its coding sequence; this suggests that the cancer may have resulted, in part, from exposure to the kind of mutagen that can produce this particular change, a GC to TA transversion.

We may be seeing here the shape of things to come. Even if mutation of the oncogene was not actually one of the rate-limiting steps in that particular patient's progression to bladder cancer, it does seem that the requisite technology is fast approaching that will make it practicable to search through selected regions of the genome for every kind of textual aberration. One spectacular example of this is about to be reported elsewhere (P. Leder, personal communication). Most B-cell lymphomas bear a translocation of the end of chromosome 8 into the region of one of the chromosomes concerned with antibody synthesis (chromosomes 2, 14 or 22). It has now been possible to isolate the region of DNA involved in this rearrangement and show that it contains the proto-oncogene called *myc*, that was originally found in association with a chicken leukaemia and, in humans, is located on chromosome 8. So here too is another human cancer arising apparently as the result of activation of an oncogene, in this case because the gene has become caught up in the active chromosomal region involved in antibody synthesis.

We seem, therefore, to be at one of those wonderful moments in science when many lines of research come together and much that was impossibly obscure suddenly seems plain. There are, however, one or two caveats that are well known to everyone engaged in this work but may not be immediately obvious to outsiders.

Natural carcinogenesis is a multistep process. Evidence for this comes from epidemiology (for example, the relation between incidence of lung cancer and the frequency and duration of cigarette smoking) and from laboratory experiments, and shows that the formation of a cancer requires several independent, rate-limiting steps. Thus, the provision of an active oncogene, whether by activation of a cellular gene through rearrangement or mutation or by viral infection, presumably corresponds to only one of a sequence of steps. Given that cancer arises from a sequence of steps, we would expect the first steps to be the ones that allow clonal expansion and free competition between cells, because this should then make all the other steps occur more efficiently and allow continuous selection of faster growing cells. But special provisions appear to have been made to prevent competition between individual cells in intact tissues. So a significant component of carcinogenesis in whole animals must be the production of whatever changes are needed in order to allow competition. If, for example, the first step in carcinogenesis has to be the liberation of cells from control by cell–cell interaction, we could not find this out by studying the transformation of cell lines which have, of course, already taken this step.

Transfection experiments with NIH 3T3 cells are open to other objections. Mice will develop cancer at the site of implanted plastic sheets, and it may be for this reason that their cells can readily be induced to grow profusely in tissue culture dishes. Guinea pigs (and probably humans) are not susceptible to such foreign body carcinogenesis, and their cells do not readily form immortalized cell lines. We could argue that the 3T3 cell is already part-way to becoming a cancer cell, and for that reason may not provide the right assay for certain kinds of tumorigenic DNA.

In transfection experiments only about one in a million cells acquire and express the exogenous DNA. This may mean that only cells already possessing a special genetic or metabolic alteration are competent to acquire foreign DNA. Such cells could be just those that have taken one of the rate-limiting steps towards cancer; if transfectibility is evidence of a step towards transformability then this step cannot be elucidated in these experiments. (Although transformation by viruses is very efficient, the viral reverse transcriptase could conceivably be making gene rearrangements much more frequent and, in that way, be by-passing what is normally a rate-limiting step.)

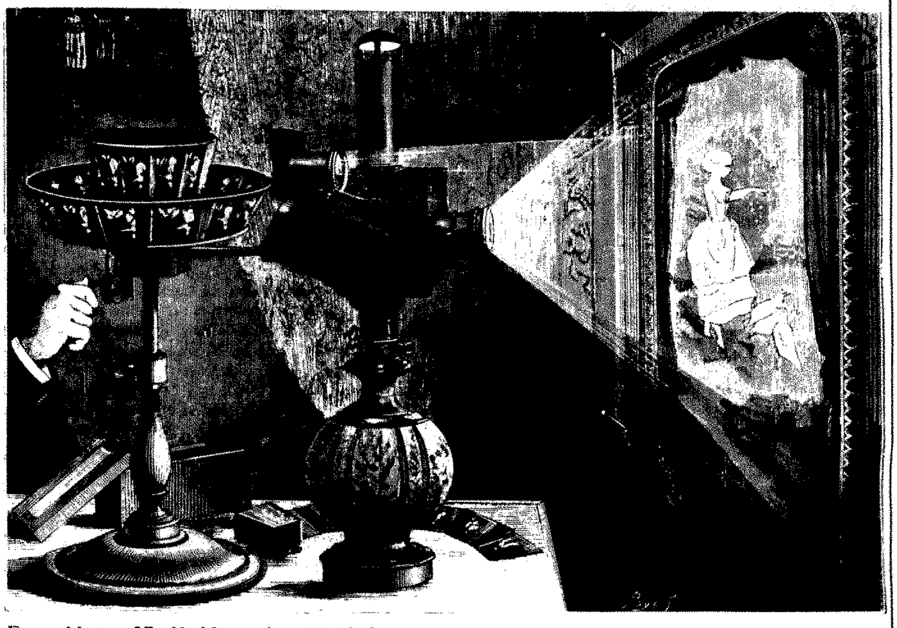
Most tumours studied so far, whether occurring naturally in humans or produced by X rays and chemicals in animals, do not yield DNA that is oncogenic in cell transfection experiments. So the assay, as presently constituted, is not detecting the genetic events responsible for many types of cancer. It is important to remember that transfection can only pick up changes that, in the genetic sense, are dominant; also it will only detect oncogenes which provide products that these particular recipient cells require for transformation (for example, the *myc* gene is not efficient at transforming NIH 3T3 cells); lastly, there is the requirement in these experiments (and in tumorigenesis by viruses) that the transforming genes are not too large to be transferred intact.

Above all, we need to keep in mind that natural human carcinogenesis is a multistep process. We do not know what the rate-limiting steps are, nor which of them are most sensitive to environmental influences and therefore a proper object for preventative strategies. But we can at least feel that the technology for studying genes and their patterns of expression are now advancing so fast that the molecular biologist will soon be telling the epidemiologist what to look for. ☐

100 years ago

THE PROJECTION PRAXINOSCOPE

M. Gaston Tissandier describes in *La Nature* an ingenious adaptation of the praxinoscope, under the above name, by means of which the images are projected on a screen, and are visible to a large assembly. Our engraving will give an idea of the arrangement and the effect produced. By a modification of the "lampascope", M. Reynaud, the inventor, obtains by means of an ordinary lamp, at once the projection of the scene or background — by the object-glass which is seen at the side of the lantern — and of the subject, by another object-glass which is shown in front of and a little above the same lantern. For this, the positions of phases which form a subject are drawn and coloured on glass, and are connected in a continuous band by means of any suitable material. One of these flexible bands is placed in the wide crown of the praxinoscope, which is pierced with openings corresponding to the phases of the subject. A hand-lever on the foot of the instrument allows a moderate and regular rotation to be communicated.



From *Nature* **27**, 61; November 16, 1882.

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The Nobel prize in physics

from Norman Dombey

THE award of the Nobel prize in physics to Ken Wilson of Cornell University honours the first of a new generation of theoretical physicists who are as familiar with the physics of thermodynamic phase transitions or the first second of existence of the Universe as they are with electron–proton scattering. This trend marks a welcome return to the spirit of the 1920s and 1930s, when the pioneers of quantum mechanics were equally at home with crystal lattices, molecules, atoms and the recently discovered nuclei. Wilson is not the only theorist to have noticed similarities between the theoretical analysis of renormalization in relativistic quantum field theory (which describes the behaviour of elementary particles) with the then unsolved classical problem of the universality of the critical indices of phase transitions between differently ordered macroscopic states of a system (such as the liquid–gas transition), but no other physicist has had the insight and stamina necessary to see the work through and to develop a general method for the calculation of critical indices.

A ferromagnet is a good example to illustrate universality. In ferromagnets, spontaneous magnetization occurs — magnetization persists in the absence of an external magnetic field, provided that the temperature is less than some critical temperature T_c. It is found experimentally that magnetization goes to zero as the temperature T tends to T_c as a power β of $(T-T_c)$. β for all ferromagnets has a value between 0.3 and 0.4. Similarly, other quantities, such as magnetic susceptibility, vary near T_c as a power γ of $1/(T-T_c)$ and experimentally $\gamma = 1.2$–1.4. Although the Ising model of a ferromagnet (see *News & Views* **299**, 675) was solved by Onsager in two dimensions to show that a phase transition exists, and can be solved numerically in three dimensions to give critical indices in reasonable agreement with experiment, it does not provide any explanation of the universality of behaviour near the critical temperature (which itself varies widely between ferromagnets).

The problem of universality can be phrased as follows: starting with an energy function describing the microscopic properties of the system of interest and containing the physical parameters of the system, for example the spin–spin interaction between neighbouring lattice sites, is it possible for the equilibrium state near the critical temperature to be independent of the initial parameters? If so, then the critical indices will depend only on the gross features of the initial energy function, such as whether the physical variable involved is a scalar or vector quantity and whether the underlying space is two- or three-dimensional. Kadanoff[2] had shown that spin-blocking (the pairing and re-pairing of spins on the lattice) provided a recursive set of transformations which could in principle take the initial energy function into the form desired.

There is an analogous result in relativistic quantum field theory. The initial parameters in the Lagrangian (the appropriate relativistic energy function) are not the measured physical parameters. This is because infinities occur in the calculational procedures and in order to remove these infinities a process of renormalization is carried out whereby the infinities are subtracted out at some arbitrary momentum Λ. Hence the physical parameters of the theory, for example charge and mass, are functions of the initial parameters and Λ, but the results of any calculation must only depend on the physical parameters and not on Λ. So a change of scale whereby Λ is replaced by 2Λ should not have any influence on the result of a calculation. Gell-Mann and Low[3] in 1954 wrote down the mathematical conditions necessary for this invariance and these are known as the renormalization group (RG) equations of the theory. Certain scaling laws among the field quantities and momenta of the theory follow from the RG equations. This is the problem that Wilson studied further throughout the 1960s.

Wilson is most remarkable in that during this period he was not at all interested in publishing papers. He received his PhD from Caltech in 1961 with a thesis[4] on a problem in quantum field theory where a relativistic pion field interacts with a static nucleon. This problem is complex enough to be interesting while tractable enough for the RG equations and scaling laws to be worked out. He did not publish the thesis and he only published one paper[5] on his progress on this problem until he obtained his main results which were published in a series of papers[6] beginning in 1969; the simplified model of his thesis played a major part in the derivation of his results. These results, very approximately, show that in a renormalizable theory at sufficiently high momenta when all masses in the theory can be neglected and therefore no basic momentum (or length) scale exists, the limiting form of the physical amplitudes are given in terms of numbers called the anomalous dimensions of the theory which are calculable using the RG equations.

Michael Fisher, who had worked for many years on the numerical calculation of critical indices, went to Cornell in 1966 from King's College, London and Wilson's awareness of the problem of universality probably stems from his presence. But it also stemmed from the field theory calculations of the fixed nucleon model in that in order to make progress with his integral equations, Wilson had split up the space-time continuum into cells, thereby giving it a lattice structure. Reversing this process, so that a continuum field theory limit could be taken starting from the crystal lattice of a ferromagnet, was a natural development.

In the region of a phase transition the correlation length in a material becomes arbitrarily large, so again there is no basic length scale. It did not take Wilson long to convert the Ising model into field theory and to look for the RG equations of the theory, albeit now in three dimensions rather than the four of relativistic field theory. For the phase transition he had to work in the low-momentum limit (long-wavelength) of the RG equations whereas in particle physics the high-momentum (short-wavelength) limit pertains. Nevertheless, anomalous dimensions were translated into critical indices and he was able in 1971 to publish a calculation of γ for the three-dimensional Ising model using the RG equations[7]. Techniques for the calculation of critical indices via the RG equations soon improved, largely due to a trick invented by Wilson and Fisher[8], and it became apparent that universality did indeed follow from the RG equations[9] since the initial parameters in the energy function were irrelevant near the phase transition.

Perhaps the most interesting lesson to be drawn from Wilson's career is that difficult problems in science deserve long study (otherwise they would not be difficult) and that solutions may well lie in methods developed in a different area of science. So extreme specialization (such as that of graduate students) and the compartmentalization beloved of bureaucrats (for example, in Britain ferromagnetism is dealt with by the Science Board of SERC while particle theory comes under the Nuclear Physics Board) may not be helpful. Another lesson is for tenure committees and for those social scientists who aim to measure successful science: it is not good enough to weigh publications or count citations. Truly original work is unlikely to

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be produced on a production line. Finally, on a hobby-horse of my own, universality, where it exists in physics, is a concept based on real experimental results, unlike the present theoretical chimaera of unification for which there is as yet no hard experimental evidence (see *Nature* 279, 675; 1979). ☐

The biology of teratocarcinomas

from P.N. Goodfellow and P.W. Andrews

THE early development of mammals is one of the major areas of biology not yet fully susceptible to the techniques of molecular biology. The chief obstacle is that the very fact of development makes it difficult to obtain enough cells, at the same stage of development, for molecular and biochemical analysis. Teratocarcinomas and their stem cells (embryonal carcinoma or EC cells) offer a solution, for these embryonic tumours display extensive cellular differentiation, often into derivatives of all three germ layers as well as the extraembryonic tissues. EC cells can be cloned and maintained *in vitro* as undifferentiated cell lines and differentiation can then be induced by altering the growth conditions, transplanting the cells into mice or by chemical inducers. Seven years ago a meeting on the biology of teratocarcinomas was held at Nutley, New Jersey[1] and progress since then was debated at a recent conference* held at Cold Spring Harbor.

Complex systems involving cellular interaction and the generation of diverse cell types can only be studied if the various cells in the system can be distinguished and if the changes in cellular phenotype are under the control of the investigator. Traditionally, morphology has served as the primary indicator of differentiation, but its assessment is subjective and it can be misleading. However, the number of biochemical and immunological markers of cellular differentiation in EC cells is rapidly expanding. These include cell surface antigens (for example, the EC marker, SSEA-1)[2], α-fetoprotein[3], alkaline phosphates[4], plasminogen activator[5], the various cytoskeletal elements (R. Oshima, La Jolla Cancer Research Foundation; R. Kemler, Friedrich-Myescher-Laboratorium der Max-Plancke Gesellschaft) and basement membrane components (C. Howe, Wistar Institute; B. Hogan, ICRF). Chemical inducers, such as retinoic acid, dibutyryl cyclic AMP and dimethylsulphoxide, permit induction of differentiation along pathways which are apparently specific for the inducer and the particular EC cell line used (M. McBurney, University of Ottawa; M. Damon, Pasteur Institute). The mechanisms by which these substances cause their effects is not known, although for retinoic acid there is an intracellular receptor, which appears to mediate the action of this agent (M. Sherman, Roche Institute of Molecular Biology, Nutley). Differentiation in culture seems also to be affected by cell contact[6,7], by growth factors produced both by stem cells (L. Gudas, Sydney Farber Cancer Institute) and by differentiated derivatives (C.M. Isacke, University of Oxford), and by interaction with extracellular matrices composed of basement membrane components such as laminin, fibronectin and type IV collagen. For example, organization of a laminin-containing basement membrane in embryoid bodies produced by suspension cultures of EC cells seems to be an important prerequisite for the outer cells to complete their differentiation to α-fetoprotein-secreting visceral endoderm-like cells (E. Adamson, La Jolla Cancer Research Foundation).

While definition of the ways EC cells differentiate in culture represents a first step, eventually it will become important to study the changes that occur at the molecular level. However, cloned DNA probes for genes regulated in early development are still a rarity. Those which are available suggest that regulation occurs, at least in part, at the level of transcription, as for the *H-2D,K* genes of the mouse histocompatibility system (G. Gachelin, Pasteur Institute). The cloning of further developmentally regulated genes is clearly a priority, and several groups are actively pursuing this area (K. Marotti, Rockefeller University; B. Hogan; C. Howe).

In the meantime, viruses remain valuable probes of cellular differentiation. Although mouse EC cells will not support expression of small tumour viruses such as polyoma or the C-type retroviruses, they can be infected by polyoma mutants which appear to be related by having suffered sequence rearrangements and mutations in the early promoter region (in the *Pvu*II-4 fragment[8]) (M. Vasseur, Pasteur Institute; E. Linney, La Jolla Cancer Research Foundation; J. Lehman, University of Colorado). The relationship between the sequence alterations and EC differentiation, however, is not clear, especially as the mutants grow normally on mouse fibroblasts. New mutants have also been isolated which are capable of infecting cell lines isolated from mid-term mouse placenta, these mutants also grow in 3T3 but cannot grow in EC cells (K. Tanaka, National Cancer Institute). At least some of the EC selected mutants can grow in the placenta-derived lines. Before these results can be ascribed to specific developmental changes it is necessary to know what cells, if any, in the adult are refractory to polyoma infection. The block to retrovirus expression is also poorly understood. The simplest explanation is that expression of virus introduced into EC or early embryonic cells (C. Stewart and R. Jaenisch, University of Hamburg) is prevented by methylation — other mechanisms must also be involved, though, for inhibition can occur before full methylation is detected (J. Gautsch, Research Institute of Scripps Clinic).

One of the exciting recent findings in molecular biology is the observation that the transforming genes of many oncornaviruses have equivalents in normal cells[9]. The possibility therefore arises that they may be genes that are important for regulating the growth of specific cell types, and may themselves be developmentally regulated. Many cellular oncogenes are indeed expressed differentially in development and several are expressed in EC cell lines (R. Muller, Salk Institute). Some of the cellular oncogenes (or at least their viral equivalents) are protein kinases which phosphorylate tyrosine[1]. It is thus interesting that several phosphotyrosine-containing proteins can be identified in early stage mouse embryos and EC cell lines and changes in them can be observed (C. Carlin, Wistar Institute).

Probably one of the most important technical innovations to be discussed at the conference was the recent discovery of a technique of deriving pluripotent EC cell lines from embryos explanted *in vitro* (E. Robertson, University of Cambridge; G. Martin, UCSF). At the earlier Nutley

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*'Teratocarcinoma stem cells' — the tenth Cold Spring Harbor Conference on Cell Proliferation was arranged by L. Silver (Cold Spring Harbor Laboratory), G R Martin (University of California, San Francisco) and S Strickland (Rockefeller University)

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meeting one of the observations reported was the ability of EC cells, transplanted into a blastocyst, to participate in normal embryonic development, leading to a chimaeric mouse with tissues derived from both the host blastocyst and the EC cell[11–13]. However, the efficiency of chimaerism with most EC cell lines is low; this may be a result of small chromosomal abnormalities of many EC cells after prolonged *in vitro* culture, although other factors may be involved since some apparently euploid lines also fail to form chimaeras (J. Rossant and M. McBurney, Brock University, Ontario). Since the embryo-derived EC cells seem to have less chromosomal abnormalities, at least in early passage, the hope is that these may produce much higher rates of chimaerism.

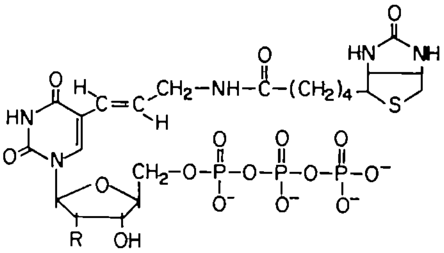
The other important possibility stemming from this technical advance is that of readily introducing known embryonic mutant genes into EC lines *in vitro*. For example, embryos homozygous for mutants of the *T/t* complex die at various stages of development. The mechanism of action of these lethal genes remains obscure, but suggestions varying from the aberrant expression of cell-surface molecules important in critical cell interactions to general metabolic deficiencies have been proposed. An embryo-derived EC cell line, homozygous for t^{w5}, which causes embryonic death at about day 8 of development, has been isolated (T. Magnusson, UCSF). However, this t^{w5}/t^{w5} EC cell line grows normally *in vitro* and forms well differentiated tumours when injected into mice. These observations suggest that embyronic lethality caused by this mutant is due to inability to organize embryonic structures at the proper time, rather than an inability simply to grow and differentiate. □

Lighting up time

from Sue Malcolm

THE report from David Ward's laboratory at Yale University School of Medicine of a new method for detecting specific DNA sequences suggests that a considerable increase in both sensitivity of detection and resolution may be just round the corner. The technique may be particularly valuable in the antenatal detection of those hereditary diseases where the molecular and chromosomal mapping of the genes involved has already been made possible. The basic idea is to incorporate a biotin-labelled nucleotide into the nucleic acid probe, to bind the substituted nucleic acid with an anti-biotin antibody (raised in rabbit) and then detect the complex either by fluorescence with a second antibody (goat anti-rabbit) coupled to fluorescein or cytochemically with the second antibody coupled to an enzyme such as horseradish peroxidase.

Present techniques rely on radiolabelling cloned DNA fragments with ^{32}P- or ^{125}I-labelled nucleotides. After hybridization, the labelled sequences can be detected with great sensitivity, but there are a number of limitations to the use of the isotopes. ^{32}P has a half-life of only two weeks and probes must be made and used immediately. This presents particular difficulties for laboratories any distance from the major suppliers (such as clinical laboratories in many of the countries where antenatal diagnosis of the haemoglobinopathies is desirable), and the handling and disposal of the radio-isotopes presents a health hazard, not to mention a bureaucratic hazard. The high energy of the radioactive emissions, particularly of ^{32}P, makes the grains on the film too scattered to give useful resolution in detecting specific mRNA sequences in individual cells, or genes on chromosomes. Tritiated nucleotides give much better resolution in cytological specimens but are necessarily also of lower specific activity, and the amount of nucleic acid corresponding to a single-copy gene on an individual chromosome or a low-abundance mRNA in a single cell is so low that their detection only becomes possible with the use of very long autoradiographic exposure times or the pooling of data.



1, R = OH
2, R = H

Biotin-labelled derivatives of UTP (1) and dUTP (2). In 1, R = OH and in 2, R = H.

The new technique makes use of biotin-labelled derivatives of dUTP and UTP (see the figure) synthesized via 5-mercurated intermediates (Langer, Waldrop & Ward *Proc. natn. Acad. Sci. U.S.A.* **78**, 6633; 1981). Other nucleotides could be synthesized by the same method but dUTP and UTP have proved the easiest to make. The Bio derivatives can be introduced easily into RNA and DNA. Bio-dUTP is a good substrate for the enzymes DNA polymerase I of *Escherichia coli* and bacteriophage T4 DNA polymerase and can be incorporated efficiently into DNA by 'nick-translation'. Bio-UTP can be incorporated into cRNA by *E. coli* RNA polymerase. Unfortunately, the analogues do not seem to function as substrates for avian myeloblastosis virus reverse transcriptase or for eukaryotic RNA polymerases.

In hybridization, Bio-substituted nucleic acids behave similarly to the non-substituted versions. There is no change in the melting temperature of DNA containing 20 biotin molecules per kilobase introduced by nick translation. Viral DNA in which all the thymidines in one strand are replaced by Bio-dUMP shows a drop in melting temperature of only 5°C. Lightly substituted DNA (5.5 per cent of thymidine substituted by Bio-dUMP) hybridizes in solution at the same rate as non-substituted DNA. The substitution may have some useful side effects — the hydrophobic side chain added to the DNA changes its physical properties and, for example, results in it being extracted into the phenol phase during phenol extraction. This could cut down background during hybridization on either nitrocellulose filters or on glass-mounted cytological preparations.

Langer-Safer and colleagues (Langer-Safer, Levine & Ward *Proc. natn. Acad. Sci. U.S.A.* **79**, 4381; 1982) have now used the biotin-labelled DNA to detect specific sequences in *Drosophila* polytene chromosomes by *in situ* hybridization. Here there are no problems of detection as the DNA in the polytene chromosomes is replicated many times. The probes were prepared with between 8 per cent and 40 per cent of their thymidine residues substituted with Bio-dUMP, and after hybridization to the chromosomes they were reacted with rabbit anti-biotin antibody. For direct fluorescence detection the slides were incubated with goat anti-rabbit antibody labelled with fluorescein and examined by phase-contrast and fluorescence microscopy. There are a few disadvantages to this method, in particular bleaching of the fluorescent signal under the light source, so a second method, using a histochemical stain, was also employed. The slides were incubated with horseradish peroxidase-conjugated sheep anti-rabbit IgG and the peroxidase activity then used to convert the substrate diaminobenzidine into an insoluble brown precipitate which can be clearly distinguished in Giemsa-stained preparations. One valuable aspect of this method is that the length of chemical exposure can be continuously monitored to give an optimal reaction time. The same method has been used by Manuelidis to detect repeated sequences in human chromosomes and mouse satellite sequences in mouse chromosomes [in *Genome Evolution* (eds Dover, G.A. &

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Flavell, R.B.) 263, Academic, New York; 1982].

The method has many potential variations, the most obvious being the use of avidin-bound enzyme complexes (to exploit the biotin/avidin K_d of 10^{-15}) or the use of biotin derivatives with longer linker arms (to increase accessibility). In fact a derivative, Bio II-dUTP, with an extra seven atoms in the linker arm, is already commercially available. The day may not be far removed when you carry your Southern blot filters to lunch with you and check their progress every now and again. □

More to melanism than meets the eye

from J.S. Jones

INDUSTRIAL melanism is the textbook example of natural selection in action. Like most such examples it is usually presented in a way which is both incomplete and inaccurate. The popular view is that a mutation to a black form appeared in the previously light-coloured populations of the peppered moth *Biston betularia* at the beginning of the Industrial Revolution. As in a sooty environment the melanics were well concealed from predators they replaced non-melanics in polluted cities while remaining at a low frequency in the countryside. The real story is, however, much more complex and a recent computer model, produced by G.S. Mani, a University of Manchester physicist, shows that selective predation, differences in fitness unrelated to crypsis and gene flow must all be taken into account before many puzzling features of melanism can be explained.

Melanism has always presented a number of problems. It appeared in industrial Britain not only in *Biston* and more than a hundred other moths, but in species not subject to intensive predation such as beetles, cats and birds[1]. In ladybirds, melanics became more common mainly because the increased efficiency of absorption of solar energy gives them an advantage in the smoky atmosphere of cities[2]. Melanic pigeons are favoured in towns because the associated hormonal changes make them better able to exploit the possibilities for winter breeding presented by the year-round availability of food[3]. In both these distantly related organisms, the mating advantage of melanic males over typicals may also be important in their evolution[4]. Although in the peppered moth the first melanic was not recorded until 1848 in Manchester, dark forms of other moths were common in forests long before the industrial revolution, and in the newly polluted cities of north-east America, industrial melanism evolved exceptionally rapidly as these variants spread from the forests. Even the apparently simple case of industrial melanism in *B. betularia* involves at least four alleles, and many British populations contain three phenotypically distinguishable classes — the pale form *typica*, the intermediate *insularia* and the full melanic *carbonaria*[1,5].

There are several puzzling aspects of melanism in *Biston*. Even in the most polluted areas, such as central Manchester, the frequency of melanics never rose above 95 per cent, although field experiments on marked individuals show that melanics are considerably better camouflaged and should have replaced lighter forms long ago if only differential predation is involved[6]. When pollution was reduced the frequency of *carbonaria* fell rapidly (from 95 per cent to 82 per cent between 1960 and 1975 near Liverpool) although the melanics still appeared better camouflaged than typicals. In addition, melanic alleles in *B. betularia* reach a frequency of 80 per cent in rural East Anglia, where there is little industrial pollution[7]. None of these observations can be explained by simple directional selection by predators.

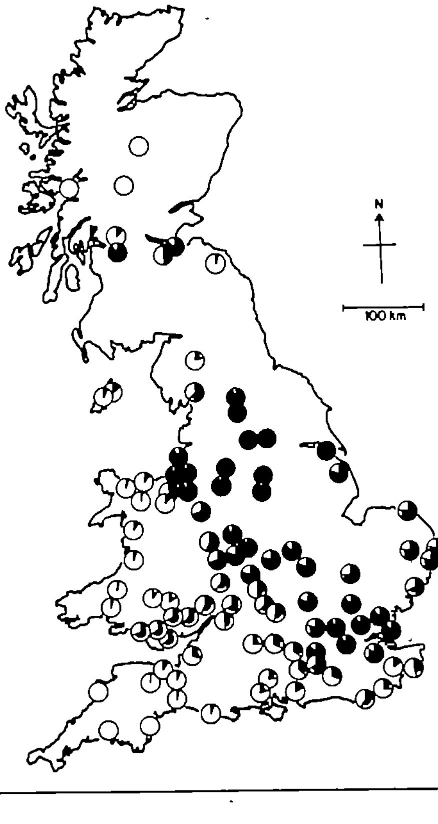

Fig. 1 Distribution of melanics in *Biston betularia*. White sector: *typica*; black sector: *carbonaria*; halved sector: *insularia* (from ref. 1).

Some of the apparent anomalies in the distribution of melanic *B. betularia* are related to its powers of migration. Males migrate about 2.5 km per generation, and this results in considerable gene flow between polluted and unpolluted places[8]. Computer simulations show that a balance between selection and migration is enough to explain the persistence of typicals in highly polluted areas[6], and partly explains why the rapid decrease in melanic frequency does not coincide with the urban boundaries of Liverpool and Manchester. However, gene flow alone cannot explain the position of this cline, nor why melanics are so common in some unpolluted parts of Britain[9].

Mani's model incorporates new information on fitness differences between melanics and typicals which are unrelated to visual selection by predators[10]. Breeding experiments involving 12,569 larvae from 83 families show such that differences are quite considerable; for example, *typica* homozygotes are at an approximately 30 per cent disadvantage to *carbonaria* homozygotes and a 7 per cent disadvantage to *insularia* homozygotes when raised in the laboratory[11]. There is no evidence of heterozygote advantage although this has often been proposed as a mechanism for maintaining melanic polymorphism. In the model, England and Wales are divided into squares with 22.5 km sides; mutation takes place at a rate of 10^{-6} per generation, and pollution in cities is assumed to have increased linearly for 55 years from 1830 and since then to have remained constant. The simulation, which incorporates experimental data on differential selection by predators, gene flow and spatially constant non-visual differences in fitness, was allowed to proceed for the 150 *B. betularia* generations from the beginning of the Industrial Revolution to the present day. It generates a distribution of melanics which is remarkably close to that found in modern populations, with an incomplete replacement of typicals even in the most polluted cities, and local patches of high melanic frequency in some unpolluted regions.

The best fit of Mani's predicted frequencies to those found in nature is obtained using estimates of non-visual

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selection which differ slightly from those resulting from breeding experiments. Nevertheless, his results make it clear that the evolution of industrial melanism in *B. betularia* must depend in part on gene flow and on powerful non-visual selective forces whose nature is as yet unknown. There remain many other problems associated with this apparently straightforward example of natural selection. Why, for example, has the frequency of *carbonaria* decreased in northern England since the introduction of smoke control, but increased in the south[7]? Even for a melanic moth it seems that life is never as simple as made out in the textbooks. □

Acetylcholine receptor cloned east and west and . . .

FURTHER to the article by C.F. Stevens (*News and Views*, 28 October 1982), it should be added that the cloning and 90 per cent of the sequence of the α-subunit of the *Torpedo* acetylcholine receptor had already been reported by a group in Britain (K. Sumikawa, M. Houghton, J. Smith, L. Bell, B. Richards and E. Barnard *Nucleic Acids Res.* **10**, 5809; 1982). The group used a two-stage procedure to design a 19-base oligodeoxyribonucleotide as an efficient hybridization probe in the cloning. From cDNA sequencing, the amino acid sequence of a 24-residue pre-N-terminal signal peptide and about 90 per cent of the sequence of the α-subunit of the *Torpedo marmorata* receptor were determined. These sequences agree with the sequence for the α-subunit of *Torpedo californica* since published by Noda *et al.* in *Nature* (28 October) and reviewed by Stevens. The only differences are at positions 42 (Asn for Ser), 230 (Val for Gly) and 318 (Asn for Asp), all single-base changes which are probably due to the species difference.

The mRNA fraction used has also been shown to be that for the native receptor by an exploitation of the oocyte translation system in which it forms the functional ion channel of the acetylcholine receptor (E. Barnard, R. Miledi and K. Sumikawa, *Proc. R. Soc.* *B*215, 241; 1982). □

Corrigendum

In the News and Views article 'A growing role for reverse transcriptase' (Nature **299**, 204; 1982), reference was inadvertently omitted to a paper by S.Z. Hirschman, S.J. Vernace and F. Schaffner (*Lancet* **1**, 1099; 1971), containing the first description of a DNA polymerase activity in preparations of human hepatitis B virus.

Pool and ridge patterns in peat mires

from Peter D. Moore

FROM the air, a peculiar pattern of concentric crescents can often be seen in the pools and ridges of a peat mire. The pattern stems from the tendency of pools on sloping mires to lengthen at right angles to the slope so that they run along the surface contours rather than downhill. But why should the peat pools grow in this manner?

Many theories have been put forward and it is possible that the causes of pool growth may differ according to the climatic environment of the mire and whether the mire surface is supplied with ground water or is dependent entirely on rainfall. Patterned peatlands are most frequent in high latitudes[1], but they occur as far south as 55°N on the western fringes of Europe to about 46°N in Minnesota[2], and they have recently been described as far south as 36°N in Japan[3].

The boreal aapa mires occupy valleys and possess ridge and pool systems which traverse their entire width. It has been proposed that they owe their structure to ice pressure from the pool systems freezing in early winter[4] but, although the freezing process could intensify a pattern once it has begun to form, it is doubtful whether this is the initial cause[1]. Snow cover may lead to a limited development of ice in winter in many Finnish mires, yet patterns are still apparent, as they are in the patterned mires of Japan, which also receive considerable snowfall in winter.

Study of Japanese mires led Sakaguchi to put forward a different theory for the origin of the patterns[6]. He claims that the initial generation of a bank/hollow complex could result from lines of accumulation of plant detritus — 'thatch lines' — carried by flood water over the mire surface. Interestingly enough the idea was considered in relation for Finnish mires in 1920 by Auer[4], but was not favoured by him. Sakaguchi backs up his proposals with observations on detritus patterns on sloping lawns after floods, which do indeed produce patterns which compare closely to those observed on a larger scale on sloping peatlands. It is an explanation which needs to be re-examined, preferably by detailed stratigraphical studies, in Finland.

Such a model is not satisfactory for the rain-fed raised and blanket mire pool patterns, for here the surface movement of water is restricted to local drainage requirements, there being no ground water supply. In these, the linear arrangement of pools again follow the contours of slope, but in this case they are usually underlain by several metres of peat and the slope itself often results from the morphological features of the peat mass. The possibility that an entire body of peat may be unstable and moving gradually down slope was proposed by Troll[7] and developed by Pearsall[8], who considered the sloping edges of a globular peat mass on a slope as a series of concentric pressure ridges.

The idea was put to the test by Pearson[9] at Muckle Moss in Northumberland. As a result of detailed surveying of posts within the peat body from a fixed position beyond the mire, Pearson was able to show that some posts had moved by as much as 3–6 mm over a period of seven years. The mass movement of the peat was thus confirmed and the pool patterns at this site could be explained in terms of ruptures resulting from this stress. The explanation cannot be applied to all patterned peatlands, however, for the pool morphology does not always conform to that expected of peat ruptures. Pools are often shallow and frequently they develop on very gentle slopes.

Boatman, Grade and Hulme[11] suggest that pools initially form in hollows which are related to irregularities in the underlying, sub-peat surface. They are then maintained by biological processes in which the peat accumulation rate is less in the vicinity of a pool than at some distance from it. The actual extent of the pools will then be related to surface water availability, one function of which is climate[12]. Some recently published stratigraphical work in Caithness, Scotland by Smart[13], shows, however, that although pool systems may have a long history, they are not necessarily persistent in the sense that they retain specific positions and expand or contract from these[14]. So pool patterns may change in the course of time as the mire succession and peat accretion proceeds. Fortunately, that very accumulation of peat contains a detailed record of the history of the mire and further stratigraphical studies are the obvious way to find out how the peatland patterns emerge. □

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AUTUMN BOOKS SUPPLEMENT

The pitfalls of bookmaking

The making of a multi-author book can be a long and frustrating business for all those involved — contributors, academic editor and publisher. Here Anthony Watkinson charts the sequence of events and describes what can go awry.

ALL RESEARCH scientists will have written papers in journals. Quite a few who have blamelessly reached middle age will be asked to write a chapter on their specialism in a multi-author volume, that is a book of newly commissioned contributions edited by a fellow scientist. All publishers meet people who will never contribute a chapter again because the experience has been too horrific. The edited book is where the publisher–author interface is raw and bleeding. What happens, and what can go wrong?

It is likely that our neophyte contributor (let us call him Campbell) will first be approached tentatively. A letter arrives from a well-known figure in his field, someone he met at a congress: Campbell is flattered, is likely to agree and then hears nothing more for months. Meanwhile the aspiring editor (Edwards, an energetic and ambitious man) has decided upon the field to be covered, has written a synopsis and picked a team of potential contributors, and is looking for a publisher. Contrary to general belief most books, like most journals, come to the publisher as ideas: few publishers are actually creative. Edwards's pitch (the form rarely varies) is as follows: the field is rapidly advancing/has reached a stage when a synthesis is desirable/has not been reviewed for 20 years (a chemist's argument this one); and although he would love to write a monograph, his commitments are too heavy/the literature is now too vast to be digested by an individual/the fractured nature of the field makes collaboration preferable.

. . . few publishers are actually creative.

There was a time when the handshake of the head of a publishing house, in the Athenaeum or over a cocktail on a roof in L.A., was a commitment enough. These days to the world outside "the publisher" is the relatively humble commissioning editor (here, Parsons). On receiving Edwards's proposal she has two tasks (the pronoun is carefully chosen on the basis of probability). First she takes advice, probably from both regular advisers and appropriate specialists. Advisers never *like* multi-authored books; they lack cohesion etc. Nevertheless, *on balance*, the synopsis appears to cover the ground, Edwards has a good reputation and, given a few judicious additions and subtractions, the proposal is worthwhile and should be accepted.

Parsons's second task is the filling-in of forms which vary from publisher to publisher, but which all aim to establish for the ruling accountants that the book will be profitable and will be profitable quickly enough. Parsons prepares a sales forecast (by imaginative extrapolation from sales of similar books) and on the same basis proposes a price. Some publishers arrive at the price first — the market price or what libraries are thought to find tolerable — and then subtract all the costs to check that the price is acceptable. Others begin by totting up the costs and then calculate the price that will produce the profit margin required by multiplying the "unit cost" (total cost divided by print number) by a certain factor, usually about six. Subtracting from the price the percentage average discount to booksellers and agents gives net income, out of which must come production costs (obtained from typesetters', printers' and binders' scales) and the royalty payment. What remains is the gross profit, which pays all the publisher's outgoings and overheads, leaving a net profit. A second calculation based on the forecasted rate of sales establishes that there will be an acceptable return on the investment within the first year or so.

In the case of Edwards's project the way ahead is now open. The book seems to be viable both academically and commercially. Parsons's final proposal is typical: the book is to be 100,000 words (300 printed pages) in length, comprised of ten chapters, and will sell at £25 with a print run of 1,500 copies. It is time to sign contracts, in which payment for Edwards and for Campbell and his fellow contributors may be a contentious point.

Almost all academic editors are these days offered royalties but contributors not necessarily. Sometimes there is a package: the editor gets a proportion and the contributors divide the rest. Other editors get the lot and make their own arrangements with their (probably medical) contributors. More common now is a royalty to the editor and a lump payment to the contributors, sometimes so much per page and sometimes a fixed sum per contribution, the publisher's argument being that the maintenance of tens of little royalty accounts is disproportionately expensive (overheads killing convenience like the problem of buying single nails). Whatever royalty is offered it is very likely to be a percentage of net income (list price less discount) not of list price: the computer is said to demand this. It used to be said — go for a royalty. However decreasing sales and lower real royalty rates nowadays mean that cash in hand is often preferable for the contributor.

Whatever the arrangement proposed, Campbell and his fellow contributors — maybe a little worried by now — should receive a contract within four months of first approach. If not, it could be that Edwards is hawking the book around less choosy publishers; alternatively, he has been told to shed a chapter or two and has not faced up to breaking the news to the contributors concerned.

Campbell's contract will be subsidiary to that between Edwards and the publisher. It may be a rather scrappy piece of paper but it must *clearly* state title and length of contribution, date by which the typescript should be delivered, who holds copyright and what reward is offered. The document is likely to bristle with misspellings, not surprisingly since in many firms the job of preparation is left to the filing clerk or an inadequately briefed contracts centre. Campbell should challenge any omission or ambiguity but it will need a mass revolt of all contributors to change the financial terms.

. . . it will need a mass revolt of all contributors to change the financial terms.

It is usually best for all concerned if copyright for a whole book is held by one body, whether publisher or learned society.

The date of delivery must be realistic: if it is less than nine months ahead no one will treat it seriously and if it is over a year the tempo is going to be very slow. Length of contribution is often vaguely expressed: does the publisher mean printed pages or A4 double-spaced typescript, and, if printed, which format ("print area" is the relevant term here, not page size)? If given in terms of number of words, does this include space needed for figures and references? In addition offprint provision is important to some — are none to be provided, or 10, 25 or even 50? These are gratis but what about the chance of buying more? Campbell should also check on free copies: he can expect one, with the possibility of buying more at a discount.

At this same time Campbell should expect to receive more than a contract. Certainly there should be notes to authors. Because publishers continue to reprint documents drawn up by long-dead production directors in the days of letterpress, demanding imperial units, World List citations and India ink illustrations on Bristol board, such anachronisms should be challenged. Academics whose drawing offices have gone in the cuts might delicately ask about in-house help or perhaps a little advance. Wizards of the SEM should check on technical points and on how many micrographs are expected.

Even more important is guidance about the aims of the book. When Campbell was first approached he is unlikely to have received a blueprint: now Edwards must set out in particular the content, the aims and the level of writing. Are references to be comprehensive (the computer search) or selective? Campbell may be asked for a synopsis to be approved and circulated: if he is, Edwards is doing his job.

In ten months' time the postman will in theory stagger up the path of Edwards's house with ten impeccably presented typescripts. In theory. Even a good editor can do little except badger the laggards with circulars and phone calls; and even a good contributor such as Campbell is forced to realize that writing chapters comes after writing papers, grant applications and lectures, and that there will be some delay: he asks for an extension of a couple of months, is given it and gets his chapter in within the year. In any one book most chapters trickle in over a period of a few months, giving the good editor time to send back for rewriting, rewrite himself, cut, argue about nomenclature, argue about facts, query the references and demand replacement illustrations — edit, in other words.

. . . even a good contributor is forced to realize that writing chapters comes after writing papers, grant applications and lectures.

If Parsons receives the complete typescript earlier than 18 months from contracts going out she will be lucky. There are always some dropouts and the chapter that is twice the length asked for, typed single-spaced, without margins and with illegible handwritten corrections, figures that no drawing office can handle and already screened half-tones. Campbell should be prepared for delays and demand the chance to update his chapter; he should also know that it is those projects which go on through several updatings over seven years or more that can kill friendships.

When Campbell knows that the typescript has been delivered in its entirety to the publisher (editors are quick to write to say this), what sort of schedule can he then expect? Book publishing like house buying is a succession of delays: before Parsons passes the typescript to the copyeditor, until the copyeditor gets to work, while queries are sent out all over the world and answers come back, after the marked-up manuscript goes to the typesetter, and so on through the proof stage (with revised proofs if needed) and on to printing and binding. Assuming reasonable efficiency, no holding back because of cash-flow problems, a prompt postal service and no industrial action in the printing industry, reckon a year from delivery of the complete typescript to publication.

Book publishing like house buying is a succession of delays.

During this period decisions have at various stages to be made within the publishing house. Before money is spent on outside suppliers (typesetters, printers etc.) Parsons has to go through the same sort of procedures as she did at contract stage but this time with a real typescript. Inevitably the extent (the length of the typescript) will be greater than bargained for however tough Edwards may have been. Typesetting and printing costs may not be much higher in real terms (production controllers in publishing houses have learnt many lessons in the past few years) but the projected print run will undoubtedly be shorter because of the poor sales record of similar titles over the same period. There will usually be two calculations to be made: first on estimates, the stage which corresponds to what the contracts call "acceptance"; and second on real costs (typesetter's and printer's bills) when the final price is set. Campbell and even Edwards will know little of the in-house agonizing. Quite frequently publishers fail to remember to tell the editor the selling price of the book.

At handover of the typescript, an unworldly academic editor will hope to be given a schedule for proofs. There won't be one. If all goes well the best hope is for proofs within six months but prior warning, let alone a schedule, is most unlikely. Production staff are surprised every year by the fact that proofs going out in the summer holiday and congress season don't get returned for months. Proofs always arrive at the most inconvenient time with an unrealistic three weeks turn-round demand, possibly reduced to a week by transatlantic postal delays. The usual system for an edited book is for contributors to return proofs to the editor, who collates them and returns a master copy to the publisher.

Suppose that the proofs are a mess. On the advice of the production department Parsons has opted for a cheap typesetter and for the money has got omissions aplenty, poor alignment of tables and an unhealthy crop of typographical errors. Campbell knows that he will be charged if the cost of making corrections is over 10 per cent of the original cost of the composition of his chapter. What can he do? He can certainly correct all mistakes that have been made by the typesetter — without fear of cost or complaint. Sometimes, however, such "mistakes" are the result of over-creative editing yet it is best to give in gracefully unless actual errors of substance have been introduced. When galley proofs were the rule authors used to rewrite in proof on the Proustian model (his galleys were famous) but the contributor of a chapter to a work of scholarship nowadays has no such chance. Each alteration means a series of actions by a highly paid operative; it can cost as much as £1 to add a comma.

Meanwhile Edwards has been contacted by the marketing department of the publisher. There is a publicity form to be filled in. The form will have asked optimistic questions about potential media coverage and the nature of the expected readership. The fact will be a badly typed regurgitation of his own summary of what the book is about, a list of places where review copies are to be sent (one hopes) and possibly requests for photocopies of the membership lists of relevant learned societies. He will not be asked about the jacket (there is unlikely to be one and the artistic design prepared by his wife may well have been lost) and there will certainly be no launching party. The best Edwards can do, poor soul, is cut down the claims (if he is a realist) and make sure that the names of all the contributors are spelled correctly.

For Campbell and his fellow contributors, the actual publication will come as an anti-climax. Edwards, being a keen editor, may have written to say that the book is "out" but his advance copy does not mean that the bookshops know about it, and it certainly does not mean that the contributors will have received their statutory free copies. Campbell, looking for reassurance, is unlikely to find it on the shelves either in the local bookshop, which received one copy on a standing order plan, or the library which bought the copy but has cataloguing delays. Publishers rarely advertise, except for a regular entry in a journal or two in which an individual title may receive much the same amount of ▶

Continued on p.137

Keeping it clean

R.C. Lewontin

Evolution and the Theory of Games. By John Maynard Smith. Pp.218. Hbk ISBN 0-521-24673-3; pbk ISBN 0-521-28884-3. (Cambridge University Press: 1982.) Hbk £18.50, $34.50; pbk £6.50, $11.95.

POPULATION genetics is the automobile mechanics of evolutionary theory. Population genetic theory is a deductive structure that asks the question: Given the mechanical details of inheritance, given mutation rates, recombination rates, migration rates, patterns of mating, the Darwinian fitnesses of various genotypes, and the relation between genotype and phenotype, how will the composition of a population or species change in time? The job of the theoretical population geneticist is to set up a mathematical machine into which the various parameters can be dumped, to turn the crank, and to produce the kinetics of evolution.

In practice this process has a number of serious difficulties. For even mildly complicated models involving several genes or non-random mating or non-constant fitness, the mathematical problems can be virtually intractable. Moreover, we do not usually know the genetic basis for traits of interest, nor are we able to measure the parameters in nature to any close accuracy.

As a consequence, evolutionists have continually searched for ways to avoid the dirty details of the kinetic equations of population genetics and to make predictions about evolution by some easier route. One form of approach has been to try to find laws of maximization or optimization, much as classical physics used Fermat's Principle of Least Action or the minimization of potential energy, in place of the kinetic equations themselves. To this class belong what R.A. Fisher called, with characteristic modesty, the Fundamental Theorem of Natural Selection, and Wright's principle of the maximization of mean fitness. Unfortunately, these maximization principles turn out not to apply generally, and the only way to know when they do is to solve the kinetic equations that we were trying to avoid in the first place.

Another attempt to make evolutionary predictions on the cheap was my introduction in 1961 of the apparatus of game theory into evolutionary biology. I had hoped that by drawing analogies — a population to the player, and the population's genetic structure and parameter values to a strategy — the entire apparatus of game theory could be used to make evolutionary predictions. But this attempt foundered on precisely the same rock as optimization theory: there is no guarantee that the actual kinetic process of gene frequency change will carry an evolving population to one of the so-called "solutions" in the game theoretic problem. That is, evolutionary equilibria are not necessarily the same as game-theoretic equilibria.

As luck would have it, I was visiting professor at Sussex University in 1971 when John Maynard Smith first began to reintroduce the metaphor of the game into evolutionary biology. At first, I was sceptical. The very words "Evolutionarily Stable Strategy" seemed to reek of the discredited attempts to avoid the messy realities of genetics. Only slowly did I come to understand that Maynard Smith was engaged in a totally different project, a project that is now lucidly explained in his new book *Evolution and the Theory of Games.* The name of the book unfortunately perpetuates a misunderstanding, for, with the exception of the metaphorical use of a few terms like "strategy" and "pay-off", Maynard Smith's theory makes no use whatever of game theoretical apparatus, but rather lies totally within the standard kinetic structure of population genetics. That is the secret of its success.

A central set of parameters for making predictions in population genetics is the set of reproductive fitnesses of the various genotypes in the population. In much of population genetic theory these fitnesses are assumed to be fixed constants characteristic of each genotype. Some progress has been made in building models that include simple dependence of fitness on population density, on the frequency of the genotype in the population, or on a fluctuating environment with known statistical properties. None of these models, however, deals with two important properties of organisms: first, any genotype may have a variety of phenotypes each with a different fitness; second, which phenotype is displayed and what its fitness is may depend upon the phenotype manifested by other individuals in the population.

This kind of doubly contingent fitness determination may make the average fitness manifested by each genotype rather difficult to compute, and so evolutionary prediction becomes very problematic. Animal behaviour in particular is full of such problems because an animal may sometimes act in one way and sometimes in another, while the fitness value of these actions will depend upon what other animals are doing. There is, then, a continuum of possible phenotypes, say from always being aggressive, through sometimes being aggressive, to always being passive, and every phenotype has a frequency-dependent fitness. And we have not even mentioned genotypes yet — not a very encouraging prospect for a theoretical population geneticist.

Maynard Smith's contribution has been to cut through this thicket by asking a somewhat more restricted question, the answer to which can be very powerful. He has developed a set of algorithms to find which of the patterns, when it is very common in the population, has a higher fitness than any other pattern that enters the population as a rare variant. The implication is that once this phenotype is established in the population, no other can displace it. Such a "King of the Mountain" phenotype is called an Evolutionarily Stable Strategy, or ESS. It turns out that such patterns exist for a wide variety of situations in animal conflict, in learned behaviour, in resource allocation and in a variety of other very complex frequency-dependent fitness problems. The real issue is whether these patterns live up to their name. Are they really evolutionarily stable?

It is at this point that the messy details of genetics reassert themselves. If the ESS pattern can be achieved by a homozygote or if reproduction is asexual, then the answer is yes. On the other hand, if the ESS pattern can only be produced by a heterozygote, then clearly it is not achievable in pure form in a sexually reproducing population. Moreover, some populations are genetically polymorphic. Can the ESS be stably created by a mixture of genotypes obeying the rules of segregation and recombination? The answer is "Sometimes yes and sometimes no". Thus, the phenotype that Maynard Smith calls ESS might better be called the "Frequently Roughly Evolutionarily Stable Strategy". A fair amount of argument in *Evolution and the Theory of Games* is devoted to convincing us that the genetic basis of behavioural phenotypes is likely to make a FRESS into an ESS. So, for example, single locus overdominance is said, probably correctly, to be rare, and it is asserted that if many genes influence a character, it is probable that any intermediate phenotype can be produced by an appropriate mixed homozygote. It must be pointed out, however, that epistatic gene interaction may be very important and that linked genes will produce a pseudo-overdominance that decays only slowly, if at all.

Other criticisms have been made of ESS theory, but Maynard Smith discounts them as being beside the point. So, predictions of ESS depend upon the set of alternative "strategies" that are evaluated. But precisely the same is true of all kinetic predictions in population genetics, which are based entirely on the alternative genotypes actually present in the population. Indeed, ESS theory has the advantage over vague optimization theories that it makes very explicit what the set of alternatives are that are being considered. ESS theory is not easy to check against natural observations because both the parameters and the predictions are usually in the form of relative frequencies that have immense standard errors, unless sample sizes are very large. But, again, that is a problem for all of population genetics, so ESS predictions are no more unfalsifiable than anything else in evolutionary genetics. The criticisms are to the point, however, when ESS theory is used uncritically, and it must be admitted that the very words "Evolutionarily Stable Strategy" have a kind of puffery about them that invites the same sort of overgeneralization as the not-so-Fundamental Theorem of Natural Selection. After all, evolution has been going on for three billion years, and if some strategy is evolutionarily stable, why surely that must mean that it is here to stay. The letters ESS now rival DNA as the most fashionable acronym in biology, and we may expect that many vulgarizers both outside and within evolutionary biology will simply not appreciate the contingency of the method.

There is a deeper sense in which ESS theory shares a flaw with all attempts to make general explanations for evolutionary events. At any moment we will see characters that are common to all members of a species, and we will be tempted to ask "Why is the species like that?". To give the answer "Because that is its optimal phenotype" is to give a totally uncheckable answer because we cannot ever know what set of alternatives was available to the species during the course of its evolution to its present state. To say that the codfish lays millions of eggs in order to maximize the number of survivors is to forget that it *might* have become ovoviviparous and so have provided maternal care. The same problem exists for ESS theory. We simply cannot say that a species is at an ESS because we do not know what set of alternatives was available. That is, such theories have predictive power but not explanatory power, *post hoc*.

On balance, ESS theory, because it is so firmly rooted in the kinetics of population genetics, must be judged as one of the more fruitful directions in theoretical evolution. Like other attempts at making generalized rules, such as the Fundamental Theorem of Natural Selection, or the principle of maximization of mean fitness, it works when the details of the genetic system are appropriate, but not always. Thus, we are still not free to ignore the material particularities that underlie phenomena. When all is said and done, "God is in the details". □

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The man who was crunched by numbers

Richard L. Gregory

Charles Babbage: Pioneer of the Modern Computer. By Anthony Hyman. Pp.350. ISBN 0-19-858170-X. (Oxford University Press: 1982.) £12.50.

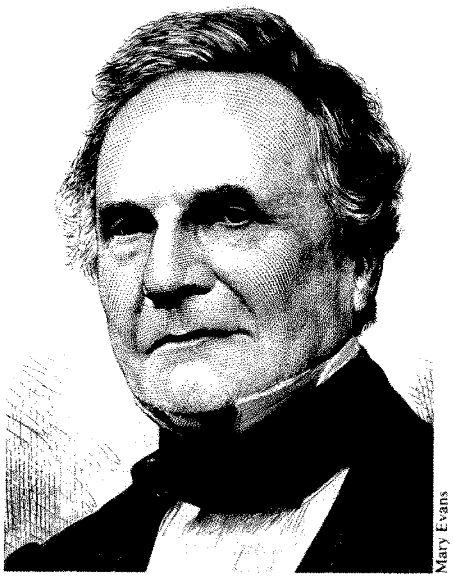
ANTHONY HYMAN'S biography of the inventor of programmable computers is no dry account of a premature technological innovation; and neither is it, as a previous biography, a shallow journalistic treatment of this truly remarkable man. Hyman has succeeded in bringing Babbage alive in the social context in which he played, as we may now appreciate, such a prominent part, and gives a clear account in just sufficient detail of Babbage's outstanding achievement, his Analytical Engine.

Charles Babbage (1791–1871) was born of a West Country banking family and became Lucasian Professor of Mathematics at the University of Cambridge (1823–1839). Elected to the Royal Society in 1816, he played a prominent if abrasive part in the scientific and technological life of his time; his influence extended to continental Europe, especially to Italy. As is well known, with advancing years he became a bitter and somewhat disappointed man; but this book shows the extraordinary range of his enthusiasms and his love of life. In retrospect he is far more to be envied than pitied, in spite of his famous defeats at the hands of Government, especially Peel's in the 1840s. Perhaps surprisingly, 20 years earlier Babbage was imaginatively supported by the Duke of Wellington when he was Prime Minister. As Hyman says (p.191):

> The underlying cause for this sad change was the developing Victorian academism. A wide gap was opening between the superior pure science of the Universities and inferior applied science, the bread and butter of the engineers Babbage's engines fell through this gap into a century's oblivion.

Those engines were only understood by a few people, among them Byron's daughter Ada, Lady Lovelace. Most difficult to grasp was the notion that in its progress towards solutions such a machine could be affected by its own findings. Wheeled calculators were known from Pascal's machine of 1642, so concepts of representing numbers and carrying out arithmetical and logical operations were fairly familiar — and indeed have been from the prehistoric abacus — though these vitally important concepts have received remarkably little comment or appreciation from philosophers; or perhaps even more surprisingly, from mathematicians or astronomers, or financial people who might have benefited greatly.

Like Helmholtz's Unconscious Inference and Freud's Unconscious Wishes, perhaps Babbage's computing engines were an unwelcome threat to human dignity; and to social and moral responsibility, for consciousness then as it still does, seemed necessary for blame and praise. Babbage's own attitude to blame and praise is convoluted. He rejected a knighthood, while also confessing to Ada that accolade and honours were particularly dear to him. He was justifiably upset that his Analytical Engine was excluded from the Great Exhibition of 1851; though he was no doubt delighted that his friend Charles Dickens based the HOW NOT TO DO IT OFFICE of *Little Dorrit* on Babbage's experience of Government's attitudes to those who have the wit to do new things.



Mary Evans

Charles Babbage, a man with a vision

Such contrasts run right through Babbage's life. He was so active in the society of the time that through Hyman's account one learns a great deal about this formative period of science and its relations with technology, as well as about Governments and how they dealt with novelty. Babbage was a man with a vision of the future industrial world as well as of computing; he was influential in the founding of several learned societies, as well as in the application of pure science in industry and commerce. He had an extremely active social life, too, being friendly with the great men of his time, and also appreciated the arts; on a splendid occasion when he was bored by a performance of Don Giovanni, he went behind the scenes and virtually invented modern theatre lighting. He invented the ophthalmoscope, and indeed everything he turned his hand to was affected by his touch. Babbage not only saw how the punch card system of Jacquard's loom could be used to control logical processes in a machine — and so how thought itself can be carried out by machinery — but, by almost superhuman effort, he developed precision engineering and mechanical drawing to a point where the general-purpose computer became very nearly if not quite a practical possibility. The full realization required the technology of electronics, but the basic concepts are there in beautifully fashioned cog wheels. (One is almost tempted to say cognitive wheels!). Babbage did as much as any man to create the machinery and the techniques — including engineering drawing — for precision mass production which has produced historically unimaginable wealth and knowledge.

All of this was achieved at the cost to himself not only of a large proportion of his personal fortune, but also in having to live with a painful gap between what he could see was needed and possible, and — for him — the stupidity and inertia of the society in which he lived. It is just this tension between his dream and our present reality that places Babbage as a man uniquely poised at the threshold of our age — leading surely to fully intelligent machines.

Anthony Hyman has succeeded in bringing the many themes of Babbage's experience and contributions together. His book is eclectic and thoughtful, as well as being both an inspiration and a warning to those of us who have faith in invention rather than received wisdom. ☐

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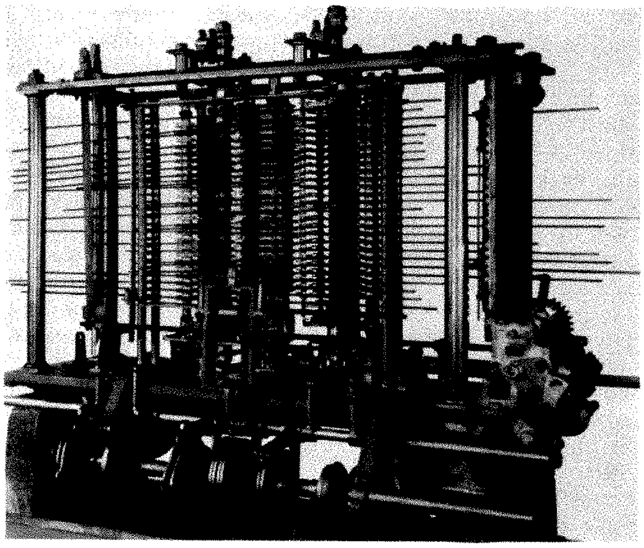

Science Museum, London

Babbage's Analytical Engine, which stands in the Science Museum in London. The machine was never completed. Babbage had thought out all of the principles on which modern computers function, but not until a century later, and the rise of electronics, could his ideas be fully realized.

Entombed in print

P.B. Medawar

A Biographical Dictionary of Scientists, 3rd Edn. Edited by Trevor I. Williams. Pp.674. ISBN 0-7136-2228-8. (Adam & Charles Black: 1982.) £15, $30.

THIS admirable book first appeared about 12 years ago and the call for a second and now for a third edition is evidence enough of its fulfilling a real need, for although it is good value it is not cheap. The innovations of the present edition are the addition of supplementary biographies of some 40 scientists who have qualified for admission — as one day we all shall — by dying, for all is in the past tense. Another valuable innovation is the inclusion of an index which under a variety of subject headings lists the names of the scientists specially connected with each one. Thus under the subject heading "Evolution" we are referred to von Baer, Darwin C, Darwin E, Dobzhansky, Haeckel, Hooker, Huxley, Lamarck, Malthus, Maupertuis, Nägeli, Naudin, Pringsheim N, Spencer, Vallisneri and Wallace — which is fair enough.

It was a claim of the first edition that it addressed general readers as well as the student of science but it really does not go very far to appease the former: the entries are baldly factual.

In this edition the grounds for inclusion of a name in the main body of the text remain as mysterious as ever (the task of deciding must have been extremely difficult). The omission of such a prominent figure as Darcy Wentworth Thompson can I suppose be excused on the grounds that he did not found a school and is not now thought of as a man who was scientifically very influential, though he was greatly admired. Clearly priority has always gone to practising scientists rather than to men who are not primarily known as or indeed known to be scientists, however great their influence on the general public, on fellow scientists or on literature or the arts.

Thus there is no place for Goethe though he did some work which he probably thought of as scientific (the "vertebral theory of the skull", for example) and his commitment to *Naturphilosophie* which exercised a wide influence — undoubtedly a harmful one. But I was sorry not to see H.G. Wells who, although he held only a fairly minor teaching post in zoology, was the author of a widely consulted zoological text and had a profound influence on the young through his brilliantly imaginative short stories which were greatly superior in all respects to those of Jules Verne. Francis Bacon rightly rates inclusion, however, because of his profound influence in throwing off the hegemony of Aristotelian thinking and propounding a philosophy of science which is recognizably congruent with modern thought even if it was in some ways pretty wrong headed. He was not much of a scientist though, and one can understand why one of his biographers called him "a medieval philosopher haunted by a modern dream".

I did not spot any errors of fact but then, I was not looking for them, taking it quite for granted that a work of such great compass and of so many hands must surely contain a few mistakes, and being unwillingly to incur the deserved odium of picking them out and calling attention to them. In the outcome I rate this dictionary as a great success and a considerable feat of editorship and of publishing which will earn the continuing gratitude of all who have occasion to refer to it. □

Sir Peter Medawar is a member of the Scientific Staff of the Medical Research Council's Clinical Research Centre, Harrow, Middlesex.

Along the lines of Einstein's thought

Arthur I. Miller

'Subtle is the Lord . . .': The Science and the Life of Albert Einstein. By Abraham Pais. Pp.552. ISBN 0-19-853907-X. (Oxford University Press: 1982.) £15, $25.

"SUBTLE is the Lord, but he is not malicious", remarked Einstein to a colleague one day in 1921. When queried further on this statement he replied that "Nature hides her secret because of her essential loftiness, but not by means of ruse". Einstein's life-long passion was to pluck from the cosmos the laws of nature ("her secret") that exist independently of the scientist as knower. And what a passion it was.

During the period from 1905 to 1916 Einstein set the foundations of twentieth century physics with two lines of research. One line dealt with invariance principles and culminated in the special (1905) and general (1915) theories of relativity. Then there were the statistical researches that resulted in a theory of Brownian motion (1905) and a route to the verification of atomic structure, the light quantum hypothesis (1905), the first quantum theory of the solid state (1907), the wave-particle duality for light (1909), and the A and B coefficients (1916) that would lead to the laser. Finally, in 1925 came one more major piece of creative work, namely his argument, independent of Louis de Broglie, for the wave-particle duality of matter.

Thematically Abraham Pais's "scientific biography" of Einstein is set out along those two lines of Einstein's research because "better than anyone before or after him he knew how to invent invariance principles, and make use of statistical fluctuations". After 1916 these two strands merged into Einstein's quest for the unified field theory, an episode in the history of science that Pais explores systematically for the first time.

Among Pais's informative biographical sections are those describing how Einstein's feats during the intensely creative period of 1905–1916 were accomplished in the face of events that would have distracted all but the most determined: he worked dutifully eight hours per day at the Patent Office in Berne (1902–1909); he moved between academic positions at the University of Zurich (1909–1911), the German University in Prague (1911–1912), back to his alma mater that had virtually disowned him in 1900, the ETH, Zurich (1912–1914), and then to the summit of the scientific world, the University of Berlin and the Kaiser Wilhelm Institute (1914–1933); he married Miliva Maric in 1903, by whom he had two sons before they separated in 1914; and he had to deal with health problems and the deprivations of war time. Little wonder that in December 1915 after completing the theory of general relativity Einstein wrote to the venerable H.A. Lorentz that he was "satisfied but rather worn out [*kaputt*]".

The episodes written with the most verve are those describing Einstein's tortuous path to the general theory of relativity and then his work towards the unified field theory. The combination of new material and infectious prose in these chapters conveys to the reader some of the excitement of doing science.

As an example consider Pais's description of Einstein's work towards the general theory of relativity. In 1913, owing to Einstein's hitherto unsuccessful research towards generalizing special relativity, Pais describes Einstein as one "who stands alone . . . possessing only a vision" of which he is "supremely confident". Let us fill in a bit. Since 1907 Einstein had been struggling to generalize special relativity to include accelerating reference systems and gravity. A key realization, Pais writes, was that he had to "start all over again". Pais argues cogently that while in Prague Einstein had done just that by using a curved space-time that required a mathematics with which he was not conversant. "Grossmann, you must help me or else I'll go crazy", he said to Marcel Grossmann his former classmate at the ETH upon rejoining him there as a colleague in 1912. There followed a joint paper with Grossmann who wrote the mathematical portion, polemics with the "fearsome" Max Abraham, and exchanges with Gustav Mie and Gunnar Nordström. These men offered alternative gravitational theories

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couched in variations of a four-dimensional flat space-time the "belief in which amounts, I feel, to something like a superstition", wrote Einstein to Erwin Freundlich in early 1914. Pais concludes that "Only Lorentz had given him some encouragement". In 1913 the doyen of German physics, Max Planck, listened to Einstein describe his work towards a general relativity theory and then told him that even if he succeeded "no one will believe you".

The onset of war does not affect Einstein's energy; the war years "rank among the most productive of his career". Amidst the tumult about him and his unsuccessful research on gravitation, in early 1915 Einstein turned to experiment in work on magnetism with Wander Johannes de Haas.

Then, in November 1915, the breakthrough occurred for the gravitation problem. He returned to the requirement of the general covariance of the field equations that he had "abandoned only with a heavy heart" in 1912. This time Einstein knew he was on the right track, because, Pais writes, "one week before the general theory of relativity was completed [25 November 1915] Einstein obtained" the correct result for the advance of Mercury's perihelion. Pais continues:

> This discovery was, I believe, by far the strongest emotional experience in Einstein's scientific life, perhaps in all his life. Nature had spoken to him. He had to be right.

There followed Einstein's prediction for the bending of light near the Sun and its verification by Arthur Stanley Eddington's Solar eclipse expedition in 1919; then was "Einstein canonized".

After 1920 Einstein left the mainstream of scientific research to pursue his vision of a generalization of relativity that unified electromagnetism and gravitation. The generalization would be based on the themes of continuity and classical causality, themes that were antithetical to the emerging atomic physics of Niels Bohr, Max Born and Werner Heisenberg. Pais asserts that Einstein had always considered a quantum theory as "provisional". He traces this strand in Einstein's thinking in great detail starting from the 1905 light quantum paper, complete with an illuminating analysis of the Bohr–Einstein dialogue of 1927–1930. Unfortunately, Pais rejects abruptly the important philosophical-physical analysis in the Einstein–Rosen–Podolsky paper of 1935 by quoting one of Bohr's *et cathedra* pronouncements of complementarity. Pais's reason, as he states straightaway in his book, is that "I would say that at his best [Einstein] was not" a philosopher.

Pais realizes that Einstein's decision to pursue a unified field theory poses a profound question that is ripe for historical analysis:

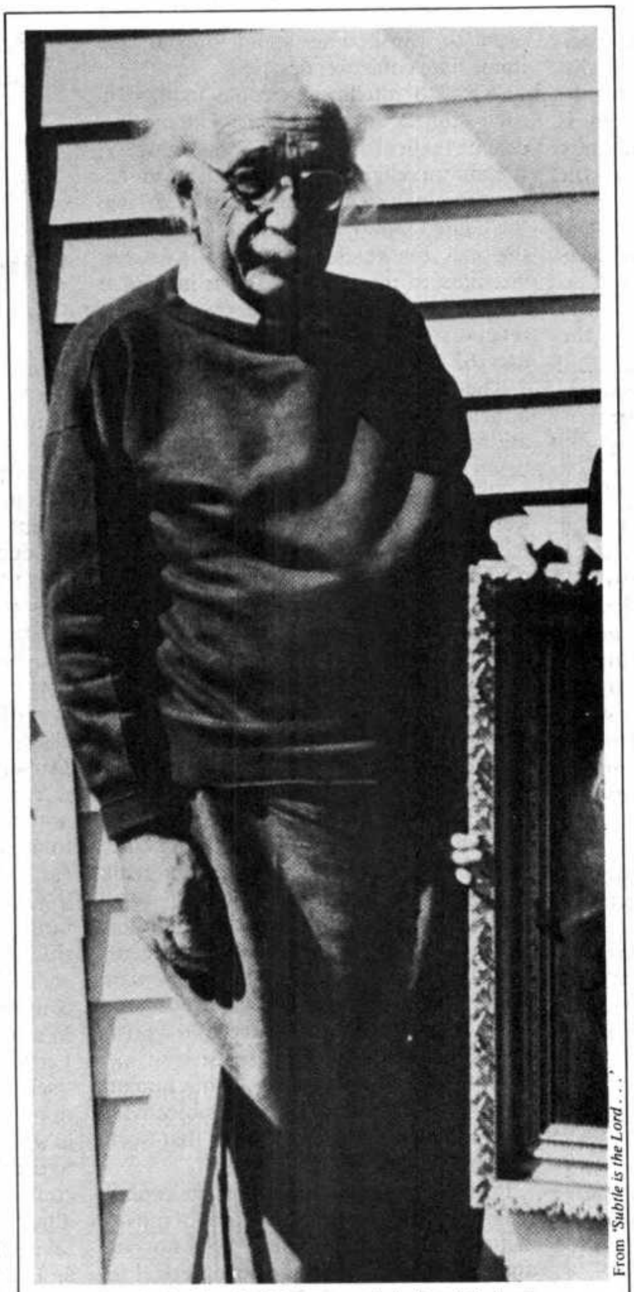
> Why does this man, who contributed so incomparably much to the creation of modern physics, remain so attached to the nineteenth century view of causality?

In outline his reply is: Einstein's infatuation with unification is evident from the beginning, as seen in his reaction to momentarily promising results from a 1901 paper where he compared intermolecular and gravitational forces — "a wonderful feeling to recognize the unifying features of a complex of phenomena", wrote Einstein to Grossmann in 1901. Although Einstein quickly abandoned the 1901 results, Pais considers that "this wonderful feeling sustained him through life". Then there was success with unification in special relativity (electricity and magnetism) and general relativity (geometry and gravity). These results, continues Pais, led him on a "quest for harmony" (geometrization of gravity and electromagnetism) based on the continuity and classical causality inherent in the differential equations of a classical field theory.

Here, and at other key points, it would have been appropriate for Pais to have developed Einstein's philosophical analysis of physical theory. Analyses of Einstein's scientific research that take seriously contemporaneous philosophical currents reveal that he was indeed a philosopher–scientist. While some lines of his eclectic philosophical view remained constant, others were transformed by his scientific research. Some investigation into this dimension of Einstein, and into other issues that are not readily apparent from the primary scientific literature, would have lent more depth to Pais's reply to a key question in the history of ideas. Pais's choice to exclude philosophical issues and his sparse use of recent historical scholarship is most costly to his analysis of special relativity theory — for example, on Einstein's invention of the theory and the theory's interpretation by Lorentz, Planck and Henri Poincaré.

Pais's forte is in the domain that he set out for himself; namely, to blend expository accounts of Einstein's complex physics with his scientific correspondence. This is no mean feat. A particularly valuable part of the book is where Pais goes on to bring research on gravitation and unification, as currently interpreted, up to date.

Abraham Pais is a distinguished physicist in his own right; his scientific biography of Einstein has clearly been a labour of love. The level of the scientific presentation is high, but so are the rewards, and the book fills in one part of the mosaic that is Einstein, his life, his times and his science. Together with such general biographical works as P. Frank's *Einstein: His Life and Times* and B. Hoffmann's *Albert Einstein: Creator and Rebel*, Pais's examination of Einstein's scientific oeuvre will be an important acquisition for both physicists and historians and philosophers of science. □


From 'Subtle is the Lord...'

Einstein in March 1955, a month before his death.

Arthur I. Miller is University Professor of Philosophy and History at the University of Lowell, and Associate of the Physics Department at Harvard University. He is author of Albert Einstein's Special Theory of Relativity: Emergence (1905) and Early Interpretation (1905-1911) *(Addison-Wesley, 1981).*

Myths of religion, myths of science

John Habgood

Abusing Science: The Case Against Creationism. By Philip Kitcher. Pp.213. ISBN 0-262-11085-7. (MIT Press: 1982.) $15, £10.50.

CREATIONISM, or "creation science", claims to provide a scientific alternative to orthodox evolutionary theory, in terms which bear a suspicious resemblance to a literal interpretation of the first chapters of Genesis. Its proponents are capable of stating their case with a display of scholarship which looks convincing to those who are not experts in biology, and their plea for tolerance, for a "balanced treatment" of creationism and evolution in school curricula, has made some disturbing headway in the United States. In alliance with the Moral Majority, creationists are now seen as a formidable threat by educationalists, scientists and non-fundamentalist theologians. As Philip Kitcher puts it "... although the Creationist campaign is advertised as an assault on evolutionary theory, it really constitutes an attack on the whole of science".

Kitcher's book is an impressive and absorbing counter-attack, which subjects all the main creationist arguments to detailed criticism. Those arguments are many and various, ranging from the accusation that evolutionary theory is unfalsifiable, to determined efforts to falsify it by appealing to palaeontology, thermodynamics, statistics, geology, uncertainties about transitional forms in the evolution of species — in fact any argument, however ancient or discredited, which seems to throw doubt on the evolutionist case. Kitcher relentlessly exposes the confusions, misunderstandings and misrepresentations on which the creationist platform is based, and provides, as he says, "a manual of intellectual self-defense" for those who find themselves under pressure. It is a splendid piece of demolition, but the effect is not merely negative. A valuable spin-off is his clear demonstration of the difference between science and pseudo-science, and this is why the book should be of interest even to those who stand outside the immediate controversies.

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Why do intelligent people indulge in such silliness as creationism? The motive, despite tactical disclaimers to the contrary, is plainly a religious one, and it is a sad fact that for many Christians, particularly in the United States, Biblical literalism is still the rock on which faith is built. Kitcher attempts to meet this problem in a final chapter by a sympathetic treatment of religious faith, but the account is sketchy and this is the weakest part of the book.

The fact that creationists link their attacks on evolution with general denunciation of atheism, communism, nazism, behaviourism, racism and every other bugbear of liberal and illiberal thinking, is an extreme expression of a much more widely felt malaise. The fear that science can be dehumanizing is not confined to cranks. Sensitive scientists have always known that they are engaged in a human and fallible activity which entails adherence to some basic human values. But popularly, and in the mouths of some of its exponents, it does not come across like that. A hard, debunking kind of science which cuts human beings and their aspirations down to size too brutally, provokes its own reaction. The cure for creation science and its like, therefore, lies deeper than the arguments mustered here, important though they are. Scientists may claim that it is not their business to take care of human self-esteem, and that in any case the theory of evolution, by showing us where we have come from, does not reduce our worth. This is fair comment. But it is also true that the refusal to face questions about human worth in the name of scientific objectivity can easily give the impression that such questions do not matter.

It is not enough, therefore, to expose religious obfuscation. The appeal of movements such as creationism will not be undermined until ordinary people feel in their bones that religious and scientific experience can be properly integrated. *Abusing Science* is a useful first step in clearing away some of the rubbish.

I have, however, one small niggle about the book. Kitcher begins by referring to the famous encounter between Bishop Wilberforce and T.H. Huxley at the British Association in 1860. This has become part of the mythology of science, but it is a gross libel on Wilberforce to classify him as a nineteenth-century equivalent of today's creationists. In his day there were good scientific arguments to be levelled against Darwinism, and the fact that he used ridicule unwisely does not affect the genuineness of the issues at stake. The point about creationism is that the arguments are now known to be worthless, so that twentieth-century religious polemic in pseudo-scientific dress belongs to quite a different moral dimension. Scientific myths, like religious ones, sometimes need to be allowed to die. □

John Habgood, formerly a Demonstrator in Physiology at the University of Cambridge, is Bishop of Durham.

Curing nonsense

Stuart Sutherland

Pluto's Republic. By Peter Medawar. Pp.351. ISBN 0-19-217726-5. (Oxford University Press: 1982.) £12.50, $25.

THE intelligensia's attitude to science is becoming increasingly ambivalent. While resenting their dependence on some of its products and fearing the dangers of others, they rejoice at the least hint that there may be something wrong with a well-established scientific theory. This propensity is encouraged by the wilful ignorance of newspaper correspondents, as witness the recent nonsensical attempt to treat cladistics and punctuation as disproofs of Darwinian theory.

Yet despite this aversion to science, nothing is more calculated to lend credence to shoddy thinking than the claim, however far fetched, that it is scientific. Psychoanalysts repeatedly make this assertion about their subject and many universities to their shame have attempted to conceal the vacuity of their social studies courses by calling them "social sciences". In a recent tome on English literature, Mr Leon Edel even claims to be practising "scientific" literary criticism. It is a pity that those in arts subjects have so little faith in what they are doing that they feel obliged to dignify it with the name of science. The confusion of the intelligensia is further illustrated by the fact that they increasingly take refuge in the gibberish of such authors as Lacan, Laing and Levi-Strauss and in such creeds as ethnomethodology, where nothing is true but thinking makes it so.

Unfortunately, few scientists have the time or the patience to expose the nonsense produced by what Sir Peter Medawar calls "the intellectual underworld", and we should be grateful to him for appointing himself a defender of reason and for untoppling some of its assailants. It is true that most of the denizens of *Pluto's Republic* are more fools than knaves, but they are none the less harmful for that.

The book incorporates two of Sir Peter's previous collections of essays, and also includes a dozen or so essays not previously published in book form. It is pleasant to reread such classic exercises in debunking as his reviews of Teilhard de Chardin's *The Phenomenon of Man* and Arthur Koestler's *The Act of Creation*, even though both books are now largely forgotten. Nothing is more difficult than exposing nonsense since one cannot refute an argument where none exists. For the most part Sir Peter exposes the authors he is attacking by direct quotation, though he cannot resist adding his own ironical asides. For example, having quoted from Teilhard "Consciousness was now leaping up and boiling in a space of super-sensory relationships and representations", he adds "The analogy . . . is with the vaporisation of water when it is brought to boiling point, and the image of hot vapour remains when all else is forgotten". He is also good at showing how a thesis leads to untenable conclusions. He points out, for instance, that since according to Koestler laughter is produced by the "bisociation" of two incompatible "matrices" of thought, the disproof of a scientific theory should produce gales of laughter. As Sir Peter remarks, "We are not amused". Irony fails him in the face of the fatuity of a passage from one of R.D. Laing's associates, David Cooper: "Curing usually implies the chemical treatment of raw materials so that they may taste better, be more useful, or last longer. Curing is essentially a medical perversion". Sir Peter's recurrent assaults on psychoanalysis are, however, more amusing than original.

His onslaughts are interspersed with the Johnsonian thump of common sense. He remarks, for example, of dreams that they may merely be noise conveying no information whatsoever, and of Levi-Strauss's insistence on the scientific validity of the Siberian myth that the touch of a woodpecker's beak will cure tooth ache: "Would not someone actually suffering from tooth ache incline towards a more pragmatic style of belief?". His common sense is also illustrated in a recent essay demonstrating that economists can never make accurate predictions: among other obstacles is the fact that the predictions may themselves influence the state of the economy. And he presents a sensible defence of genetic counselling: "Scientific evidence bears on decisions which are not of course themselves scientific".

He also resembles Dr Johnson in his capacity to write with an air of authority even — as occasionally happens — on topics he seems to know little about. For example, he asserts that intelligence cannot be measured by a single number and that there cannot be such a thing as a "culture free" test of intelligence, but he makes no reference to the extensive research on both topics.

About a third of his new book is devoted to the philosophy of science. Although these essays are a little repetitious, Sir Peter provides a clearly stated summary of Karl Popper's position, to which he has added with considerable historical erudition an account of the work of some of those, such as Whewell, who anticipated Popper many years ago. One cannot help wishing that Sir Peter had taken up his sword to destroy some of Popper's apostates such as Feyerabend, whose confusions are as much in need of exposing as those of Teilhard or Koestler.

Although not everything Sir Peter has to say is original, it is always well said. It is a truism that among academics scientists write better than those in arts subjects, but he writes exceptionally well even for a scientist. He is never short of an apt quotation, a nicely chosen simile ("They accepted the contemporary and far from adequate form of Darwinism in much the same way that nicely brought up people accept their religion, that is, in a manner that contrives to be both tenacious and perfunctory"), or a witticism ("Haldane was totally lacking in worldly sense, a sulky innocent, a whole-hearted believer in *Them* — the agents of that hidden conspiracy against ordinary decent people, the authorities who withheld the grants he had never asked for and who broke the promises they had never made").

Bern Schwartz

Sir Peter Medawar — the Johnsonian thump of common sense.

Sir Peter has invented a literary genre of his own, which might be called *belles-sciences*, though he is perhaps at his best when he is exposing nonsense, of which there is still an abundance. If he thought *The Act of Creation* boring, let him try reading Lacan. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition at the University of Sussex.

Nuts about PK

Martin Gardner

Psychokinesis: A Study of Paranormal Forces Through the Ages. By John L. Randall. Pp.256. ISBN 0-285-62540-3. (Souvenir Press: 1982.) £8.95.

MANY worthless books by writers who call themselves parapsychologists have been published in recent years, but *Psychokinesis* by John Randall, a biology teacher at Coventry School, tops them all. It is not just that he rakes over stale ground but that his book is hopelessly out of date.

Consider, for example, his enthusiastic endorsement of the psychokinetic (PK) powers of Uri Geller, the Israeli magician turned flim-flam artist. Randall makes much of John Taylor's high praise of Geller, and of the spoon-bending children featured in Taylor's book *Superminds* (Macmillan, 1973). He never informs his readers that in 1978 Taylor executed a 180-degree turn and denounced all psychic metal bending as fraud. Taylor even wrote a book about his disenchantment: *Science and the Supernatural,* published by Dutton in 1980.

Consider the pages in which Randall rhapsodizes over Geller's alleged alteration of the chemical structure of a piece of nitinol wire, giving it a new "memory" which experts could not remove. That is totally false. Eldon Byrd was in error when he made this sensational claim, reporting on his nitinol tests with Geller in Charles Panati's now discredited anthology, *The Geller Papers* (Houghton Mifflin, 1976). Experts at the Lawrence Livermore Laboratory, in California, removed the wire's memory easily. This was carefully detailed in my paper, "Geller, Gulls and Nitinol" (1977) (reprinted in *Science: Good, Bad and Bogus*), but either Randall never read it or, what is worse, read it but did not want to mention it.

Consider too the section in which Randall extolls the "thoughtography" of Ted Serios, a Chicago bellhop, who for a short time was apparently able to project onto Polaroid film his memory of photographs he had seen earlier in magazines. No one, says Randall, ever found evidence of trickery. Nonsense. In 1967 Charles Reynolds and David Eisendrath published in *Popular Photography* a complete exposé of how Serios performed his whimsical trick. Ted has been unable to replicate it since, although magician James Randi demonstrates it regularly and with more skill. Did Randall know of this exposé? If not, his research was amateurish. If he did, his failure to discuss it is reprehensible.

The sad fact is that Randall buys almost everything on the psi scene no matter how flimsy the evidence. He believes the PK is behind the spirit rappings and the table liftings produced by great mediums of the past. Many pages describe the levitations of human bodies from early Christian saints to meditating yogis; Randall suspects that PK helped Nijinsky make his high leaps on the ballet stage! He is persuaded that PK can hurl heavy objects across a room, move the hands of a clock and cause light objects, such as matches and pill boxes, to "walk" across a table.

A postscript summarizes the 1981 "minilab" phenomena recently disclosed by W.E. Cox, a former associate of J.B. Rhine and a confirmed Gellerite. Leather rings link and unlink. A pencil rises in the air and writes on a notepad. Randall assures us that Cox captured all these wonders on film, but he doesn't reveal that the films are peppered with time breaks, and that Cox's efforts to prevent fraud were so minimal that when he presented his stop-action films at a parapsychology conclave last year in Syracuse, New York, they were greeted with derision.

Randall even thinks PK is behind an old parlour trick in which four people use extended little fingers to hoist a heavy person from a chair. Samuel Pepys describes it in his *Diary* as a pastime of French schoolgirls. Geller does this stunt often in his stage act, using a fat man from the audience and three volunteers to assist him in the lifting. (By including himself among the lifters Geller can make sure the lift fails on the first attempt when PK forces are not invoked.) Hilarious photographs of Colin Wilson being "levitated" in this manner are in his preposterous book, *The Geller Phenomenon* (Aldus Books, 1976). "As far as I know", writes Randall, "no physicist has ever given a 'normal' explanation of this phenomenon".

What is there to explain? The first lift is uncoordinated. When it is repeated, after a preliminary ritual of some sort, the four lifts are synchronized, the weight is thereby evenly distributed four ways, everybody tries harder and up goes the fat man.

Randall believes everything. Well, not quite. He is surprisingly sceptical about what parapsychologists call "animal psi". When Helmut Schmidt's cockroaches seemed to use their PK to bias an electronic randomizer, Randall suspects it was not cockroach PK that did it, but PK from Schmidt or one of his assistants. Randall himself has pubished papers on how an experimenter's PK can influence animal life; in one case it modified the paths taken by crawling wood lice, in another test, the direction in which gerbils jumped. He was unable to replicate the positive results of a 1952 experiment by Nigel Richmond in which Richmond's PK influenced the paths of swimming paramecia.

To explain PK Randall dredges up an old theory, popular with Spiritualists a hundred years ago, that psychics somehow have the power to shift matter in and out of hyperspace. This was the opinion of a German physicist Johann Zöllner, to whom Randall devotes an entire chapter. Zöllner used the theory to account for the sensational results of his experiments with the famous American slate-writing medium, Henry Slade. His book about Slade, *Transcendental Physics* (English translation, 1880), is almost as funny as Jule Eisenbud's *The World of Ted Serios* (Morrow, 1967). Randall thinks I was unfair to Zöllner when I once called him "a remarkably stupid fellow". On the contrary, Randall is convinced that Zöllner was an astute investigator of Slade's genuine powers.

Randall's bibliography of 193 references is notable only for its omissions. Where, for example, is Edward Girden's classic paper, "A Review of Psychokinesis" (*Psychological Bulletin* **59**, 353–388; 1962)? In view of Randall's claim that magicians have no idea how Geller does his tiresome tricks, why does he neglect to list two books on sale in magic shops, *Confessions of a Psychic* (Karl Fulves, 1975) and *Further Confessions of a Psychic* (Karl Fulves, 1980), both by Uriah Fuller, which give Geller's techniques in full detail?

Uninformed readers may find Randall's book persuasive, but most professional parapsychologists will be as embarrassed by it as they are by the scribblings of such irresponsible journalists of the occult as Colin Wilson, Lyall Watson and D. Scott Rogo. *Psychokinesis* is only the latest, but surely not the last, of a seemingly endless line of lurid books about the paranormal, hacked out by gullible believers who are incapable of distinguishing competent investigations from shabby research and anecdotal poppycock. ☐

Martin Gardner is a science writer, formerly with Scientific American *and now a regular contributor to* Discover. *His most recent books are* Science: Good, Bad and Bogus *(Prometheus, 1981) and* Aha, Gotcha! *(W.H. Freeman, 1982).*

Neotony: is the child father to the man?

P.E. Bryant

Growing Young. By Ashley Montagu. Pp.306. ISBN 0-07-042841-7. (McGraw-Hill: 1981.) $12.95.

It is easy to detect a certain general ambivalence about the significance of childhood. On the one hand children seem to have some admirable and quite distinctive talents: they learn, for example, some very complicated things such as language (and sometimes two or more languages) with astonishing ease and they impress us with their quickness and their inventiveness. But on the other hand child psychologists keep on telling us that children lack many essential and therefore desirable qualities, and indeed these professionals devote much energy and ingenuity to the goal of hurrying on children's "development" and making them more like adults as quickly as possible.

Several people seem to manage to take both points of view at the same time even though the two obviously contradict one another. Ashley Montagu is not one of these people. His book is devoted unambiguously and enthusiastically to the proposition that childish qualities are not only desirable: they are, he thinks, precisely what distinguishes us from other species.

There are two parts to his thesis, the first anatomical and the second behavioural. The first part is the more familiar of the two and it also has more empirical support than the second. Neotony, a theory originally put forward by Bolk and lately extended in a well-known book by Stephen Gould (*Ontogeny and Phylogeny*; Harvard University Press, 1977), is Ashley Montagu's starting point. He takes up the argument that adult human beings preserve more of the physical features which characterize their childhood than do the adults of other species. Moreover, he argues, childhood takes up a larger proportion of the life span of humans than it does of other species. This, as far as the author is concerned, is not just a quantitative difference. He dwells on the evidence that human babies are born at an early gestational stage, and suggests that this is a significant factor in the quality of human social relationships. His argument is that much of family life is influenced by the need to protect and nurture helpless human infants.

Ashley Montagu — enthusiasm for neotony.

Having made this case persuasively and clearly the author then tries as well to make a direct connection with behaviour, and here he is notably less successful. His theory is that neotony is not just a matter of anatomy; human behaviour too, he claims, is strikingly "neotonous". The author attributes man's evolutionary success to the retention of traits which are characteristic of human childhood. These so-called "neotonous traits" include curiosity, creativity and sensitivity. Ashley Montagu's idea is that these are childish things which adults, and particularly those who are successful or at least admirable, retain through their lives.

There are several things wrong with this second part of the book. One is simply that the author rarely produces anything like convincing evidence for his thesis. There never has been any evidence at all that children are more creative than adults, for the very good reason that we have never had any good measures of creativity. The author's discussion of what pathetically unconvincing measures there are is strikingly uncritical and not even up to date. But his case is even less compelling when he talks about sensitivity, or flexibility or optimism (all of them put forward as neotonous traits), for here he can produce no evidence at all. Too often he is reduced either to stating his own unsupported impressions or to quoting the equally unsupported opinions of other theorists.

Another problem is that Ashley Montagu's (always attractive) enthusiasm for his neotonous case is so strong that he seems unable to notice that his thesis is, as far as several other people's theories are concerned, an antithesis. He gaily quotes Piaget, and yet apparently fails to notice that Piaget spent his whole working life trying to prove the startling intellectual inadequacies of childhood. The contrast between Montagu's optimism and Piaget's pessimism about childish behaviour is stark indeed: I cannot see how they could both be right. But there is no reason why Montagu should be worried by this, since there is now plenty of evidence that Piaget's gloomy stance was, to say the least, exaggerated.

This leads us to another difficulty about the book. Not only does the author skirt round possible arguments against his thesis; he also ignores a great deal of evidence which could be used to support it. The last ten years or so have seen a series of extraordinarily striking experiments which apparently demonstrate that even very young children have intellectual capacities which had been previously thought to come much later on in life. Since this research seems to show how sophisticated and effective even very young children are, it really is right up Ashley Montagu's street. Yet he all but ignores it. So we are left with a book which may indeed be right, but right often for reasons which the author himself leaves out. ☐

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In between ethology and neurophysiology

R.M. Seyfarth

The Human Primate. By Richard Passingham. Pp.390. Hbk ISBN 0-7167-1356-X; pbk ISBN 0-7167-1357-8. (W.H. Freeman: 1982.) Hbk $19.95, £14.95; pbk $10.95, £7.50.

A GOOD science book, one is told, does not just organize and summarize results: it presents a novel synthesis of previous work, offers new hypotheses and points the way to new areas of investigation. This is a difficult task in any field, but it promises to be particularly frustrating when the chosen topics are evolution, the brain, intelligence, and language in human and non-human primates. Not only is this research scientifically complex, but it also has uncomfortable Cartesian overtones, since in the long run it aims to establish a boundary between human beings and other animals. Moreover, there's Catch-22. The more we find human features in the brain or in the communication of non-human primates, the more these animals become ideal models for research on human pathologies. At the same time, increasing evidence of similarities between human beings, apes and monkeys raises serious ethical and scientific questions about the conduct of such laboratory research. The ethical questions are being debated — for example by Marian Dawkins in *Animal Suffering: The Science of Animal Welfare* (Chapman & Hall, 1980). Now Richard Passingham raises some of the scientific issues that are equally worthy of consideration.

How should we characterize a brain that is so much like our own and yet clearly different? Where do the intellectual talents of monkeys and apes end and our own unique abilities begin? Is it wise, given the extraordinary anatomical similarities between ourselves and other primates, to assume a quantal difference between our language and their communication? In addressing these issues Passingham sets himself a considerable task, and it is a testament to his efforts that he partially succeeds.

In my view, the least interesting chapters are those on non-human primate relatives, technology, and culture, in which the author simply reviews areas that are traditionally covered in most biological anthropology textbooks. There are other chapters, however, where the material itself is not new but where the author juxtaposes studies that have not previously been treated together. In Chapter 5 Passingham nicely connects laboratory studies of animal intelligence (usually found in psychology textbooks) with field research on the same species (usually found elsewhere). Similarly, in Chapter 6 he tries (with less success) to link the laboratory "ape language" projects with research on the communication of primates in their natural habitats. Although such field and laboratory studies seem obviously related intellectually, *The Human Primate* is, surprisingly, one of the first books to treat them together.

This said, novel syntheses can be frustrating because they often reveal more about biases and failures than about insights and successes. Take intelligence: here Passingham reviews the correlation between performance on a variety of psychological tests and at least one index of brain development. To no one's surprise, monkeys and apes score better than other mammals. Insufficient emphasis, however, is placed on the fact that as yet we have no test that reliably distinguishes between the skills of apes and of Old World monkeys. Somehow we *know* that chimps are smarter than macaques, but thus far comparative psychologists have been unable to describe what this actually means. Even when species differences in test performance are found, no successful effort has been made to determine the relation between such scores and the animals' natural social behaviour. For instance while Clutton-Brock and Harvey have shown that primate species with larger brains have larger home ranges, to those interested in how intelligence works and has evolved this remains nothing more than a tantalizing correlation.

Similarly, in other sections Passingham synthesizes two studies on one page, only to adopt a page later the same narrow perspective that has kept them apart for so long. In comparing the "ape language" studies with research on the natural signals of chimps, he correctly notes that evolution is unlikely to have supplied an animal with communicative skills for which it has no need. Thus we should expect, if only as a working hypothesis, to find that free-ranging chimps or other primates are capable of using sounds or other signs to represent objects in the world around them. Passingham concludes too readily that there is no such evidence. The fact is: no one has really looked. For while the ape language projects have begun on the assumption that their subjects are capable of at least rudimentary semantics, observers of the same or related species in the wild have generally not even considered this as a possibility. Given such biases, it is not surprising that few parallels have been found between primates' abilities in the laboratory and their natural communication in the wild. But it would be premature to conclude that no such continuities exist when, until recently, they haven't even been considered.

The Human Primate makes its most important contribution, and has the most to say about the future conduct of research on non-human primates, where it addresses studies of brain mechanisms,

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language and communication. Consider, for example, the search for neural lateralization in non-human primates, or for non-human primate analogues of Broca's and Wernicke's areas in the human brain. Here Passingham's lucid review makes one aware that most research thus far has been a curious combination of highly sophisticated neurophysiology and behavioural assays that are crude and primitive by comparison. When the subjects are human beings, every effort is made to see that they live as normal lives as possible, and that they are tested with meaningful stimuli like words, pictures and other conspecifics. In contrast monkey subjects are often kept in isolation cages and tested with tones, hisses and human speech. Under these conditions is it any wonder that parallels in brain function are often hard to find? Passingham's review offers a challenge for future research, because it shows clearly that good neurophysiology requires equally good ethology. In order to understand the monkey's brain we must also understand — and respect — the social environment in which it has evolved. ☐

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Race is everything

W.F. Bynum

The Idea of Race in Science: Great Britain 1800–1960. By Nancy Stepan. Pp.230. UK ISBN 0-333-28856-4; US ISBN 0-208-01972-3. (Macmillan Press, London/ Archon (Shoe String Press), Hamden, Connecticut: 1982.) £20, $27.50.

IN LIBERAL circles nowadays, "race", if not exactly a dirty word, is at least one which can be vaguely embarrassing. Modern anthropology stresses social forms rather than biological natures as central to the differences between "us" and "them". This was not always the case. As the Scottish anatomist Robert Knox wrote in 1850, "Race is everything: literature, science, art — in a word, civilization, depends on it". To be sure, Knox was a little bit suspect even in his own time, since his religious views were heterodox and he had been the prime recipient of the merchandise purveyed by Burke and Hare. But Knox's racial theories were part of the nineteenth-century fascination with the subject, and not simply as it related to the variations between Hottentots and Englishmen. Anthropology could be studied closer to home: in the differences between the Saxon, who built his detached home in splendid, self-reliant isolation, and the Celt, who swarmed in the slums of the teeming Victorian cities. Norman yokes and Aryan races were part of the substance of nineteenth-century science.

The title of Nancy Stepan's new book is significant: *Race in Science.* Concentrating primarily on Britain, she traces the rise of the scientific idea of race in the eighteenth century, through its burgeoning in the next century, to its decline in our own. The peripheries are rather fuzzy, for there was a preoccupation with the nature of human differences long before the Enlightenment, and the modern legacy of old-fashioned racial studies (what Stepan calls the "new science of human diversity") is not without its historical reverberations. Not ten years ago Britain's oldest university press published a massive book by a Fellow of its oldest scientific society entitled simply *Race.* Nevertheless, Stepan insists, the

> tradition of scientific racism which had lasted from the early nineteenth century until the end of the Second World War if not later, has at long last been laid almost to rest.

The phrase "scientific racism" raises issues which, as we shall see, Stepan is conscious of but does not resolve. But what she does, soberly and cogently, is to describe the idea of race in Britain during the past two centuries. Her book is based on an intelligent reading of the relevant primary and secondary literature, and the result is a thoughtful, well-written synthesis. Three principal themes successively dominated debates on race during the period Stepan covers: the original unity or diversity of mankind; man's evolutionary origins; and the relative eugenic worth of the various human races.

Traditional theological and scientific orthodoxy asserted that all human beings are descended from a single pair, created by God and placed in the Garden of Eden. In the nineteenth century, this monogenist view was most authoritatively developed by the Bristol physician and ethnologist James Cowles Prichard (1786–1848). In a series of erudite books, culminating in the five-volume, third edition of his *Researches into the Physical History of Mankind* (1836–1847), Prichard marshalled the anatomical, physiological and linguistic evidence favouring the original unity of man. His Christian vision inspired many of the founders of the Ethnological Society of London (1843), but by the time of Prichard's death a more secular, continental-inspired polygenism (as represented by Knox) was rapidly gaining ascendency in British circles. This mid-century polygenism was rooted in the notion of the biological *type* and stressed the permanency of inheritance at the expense of the more fluid environmentalism of earlier naturalists.

After 1859, of course, Darwinian evolution and the contemporary discovery of man's antiquity gave a broader framework for theories of the origin of human beings and human races. Curiously enough, as Stepan correctly insists (echoing a point persuasively argued by the historian of anthropology George W. Stocking, Jr), late nineteenth-century debates were often couched in roughly the same terms as pre-Darwinian ones. Polygenism persisted long after evolutionary biology might have been expected to have made its traditional formulation irrelevant. Part of the intellectual reason is that typological thinking can be neater and more satisfying than the population or statistical thinking inherent in Darwinian definitions of species and in the Darwinian vision of nature.

Statistics, however, was also central to eugenics, named and inspired by Darwin's cousin Francis Galton. In Britain, eugenicists were generally more concerned with social class than with race, although Stepan deftly picks her way through the eugenics movement, focusing on the all-too-easy transition from class to race. Both eugenics and racial biology in Britain were profoundly affected by the revulsion to Nazi ideas and practices, and the establishment of the neo-Darwinian synthesis by Ronald Fisher, J.B.S. Haldane and Theodosius Dobzhansky gave new sophistication to population genetics at the expense of the typological approach. Since the Second World War, the main arena for these debates has been the psychological laboratory rather than the anthropological field station, as the nature–nurture polarity has revolved primarily around intelligence. In the United States, which at least until the recent past has had a more turbulent racial history than Britain, Arthur Jensen's researches have racial overtones; in Britain, Cyril Burt's data were aimed at showing class differences in intelligence.

Stepan discusses all these issues and more in a book which has many virtues. What she does not do, however, is explore analytically the implications of one of her explicit working assumptions:

> that though the connection between racism and science is inescapable, the story of scientific racism is not merely a story of "pseudoscience". Bad science, perhaps, but not pseudoscience [p.xvi].

Her work hints at, but never comes to grips with, the relation between "science" and "prejudice", and between "good" science and "bad" science; with the nature of scientific knowledge; or with those wider issues of class, sex and empire, fact and value, which are central to that telling phrase, *scientific racism*. To write such a book would be vastly ambitious, but it should be possible even within Stepan's own liberal historiographical framework. In the meantime, her present book deserves to be read and pondered by anyone interested in the human sciences or their history. ☐

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Theoretical archaeology in Melos

Peter Warren

An Island Polity: The Archaeology of Exploitation in Melos. Edited by Colin Renfrew and Malcolm Wagstaff. Pp.375. ISBN 0-521-23785-8. (Cambridge University Press: 1982.) £35, $65.

From 1974 to 1977 the Greek island of Melos was the subject of a multidisciplinary environmental survey, the fieldwork being closely integrated with excavations at the great Bronze Age site of Phylakopi. An account of these excavations, directed for the British School at Athens by Professor Colin Renfrew, will be published separately. The present volume has as its basis the publication of the survey. There are 15 contributors, J. Cherry, C. Renfrew, B. Sparkes and M. Wagstaff writing major parts. Most of the team involved are or were at the University of Southampton and the volume must certainly rank as a major research achievement of that institution.

The project was concerned with the economic, social and political history of Melos, from the Neolithic period to the present, in relation to the island's whole environment as a resource for exploitation. Geology, geomorphological evolution, agriculture, animal husbandry and mineral exploitation are discussed in detail. The mineralogical studies include reference to millstone extraction, Hellenistic and Roman mining of sulphur and other minerals, and a detailed account by R. Torrence of the celebrated obsidian sources. Her statistical analysis of waste and worked pieces indicates unsystematic exploitation of the sources, with no evidence that Phylakopi controlled an Aegean market economy in obsidian.

Other contributions examine settlement distributions, forms and history (together with an excellent site gazetteer by Cherry), and the changing impact of autonomy and external control on the Melian populations. There is some fine topographical work by Cherry and Sparkes on the Classical town of Ancient Melos. Wagstaff has a most informative chapter on the relations of mediaeval Melos with the outside world, with invasion maps of Arab and other raiders worthy of a central European *Völkerwanderungatlas*. The same author and S. Augustson examine traditional land use in the island; their interesting data for plot distances and journey times from farms and homes suggest controlling factors specific to Melos, or at least in contrast to those for Crete and Messenia. It would have been helpful if the land-use questionnaires had been included in the book, though the authors do provide much statistical information on the calculation of labour intensity coefficients for agriculture. The text throughout is supported by an extensive range of maps and tables.

Today all this is expected of such a project in early Aegean cultural studies. The account of the University of Minnesota's work in Messenia, *The University of Minnesota Messenia Expedition: Reconstructing a Bronze Age Regional Environment* (1972), is a magnificent predecessor. Current work in the southern Argolid and in Boeotia by American and British teams respectively is on similar, large-scale, multidisciplinary lines.

But here all comparisons stop. The present volume is *sui generis* in Aegean studies. It has a character and purpose far beyond the achievements of the studies so far referred to. The guiding inspiration is Professor Renfrew's. The purpose is not simply environmental reconstruction and explanation of state formation and disaggregation through time; rather it is to use Melos as an instance of the general processes affecting modes of state formation. So the character of the book is largely theoretical, deploying a wide range of models to investigate the central aim.

The living past on Melos — threshing the harvest in the traditional manner, using two donkeys to pull the threshing sledge (*volosyro*).

At times the text is not easy to follow: detailed modelling of the "intensification of subsistence production" in terms of the "production function" and "the incentive elasticity of labour input" are crucial concepts. A model of "socio-spatio-information hierarchy" for Melian state formation is discussed but rejected. So too are explanations based on irrigation, redistribution, secondary state status, importation of prestige goods and a temple-based system. The explanatory model that is chosen is one of "peer polity interaction". This answers well to *growth* in the economies and societies of the Aegean city states, growth through continuous economic exchanges between the states, from their differing resource bases. But I am not sure that it explains *initial* state formation. In Renfrew's final chapter the foundation of Classical Melos by Dorians emigrating from Lakonia, discussed earlier in the book, is strangely neglected.

My own view, which I am confident by no means all Aegean archaeologists will share, is that this book will have an important (and perhaps divisive) influence on future Aegean studies which attempt to go beyond the descriptive and try to tackle economic and social questions. It therefore merits long and serious study, even if one feels that what are proposed in the introduction as general propositions and hypotheses which "have dictated the final form of the book" seem either so general as to be unhelpfully obvious (resource potentials will support a range of population densities) or look rather like inferences after the fieldwork was done. But any big field project will develop its aims as it goes along; it responds to observed data. There is certainly no harm in that. ☐

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Politics, personalia and the past

Jonathan Tubb

Digging for God and Country: Exploration, Archeology, and the Secret Struggle for the Holy Land 1799–1917. By Neil Asher Silberman. Pp.228. ISBN 0-394-51139-5. (Knopf: 1982.) $16.95.

THERE are many people whose radios never change from a station broadcasting popular music yet will avidly read books on the life of Vivaldi or the extra-marital affairs of Alban Berg and will derive great enjoyment from them. The point of making this statement is simply to emphasize that it is not necessary to be a Palestinian archaeologist, or for that matter an archaeologist of any sort, to appreciate Neil Asher Silberman's remarkable book.

Unlike other authors who have charted the progress of exploration in the Holy Land, Silberman has gone much further, not only chronologically by extending the story back to the exploration by Napolean Bonaparte's "Scientific and Artistic Commission", but also in manner of presentation. He has avoided a tedious procession of facts about who dug what, where, when, and what they found, and instead presents a series of fascinating insights into the various researches seen in the context of the prevailing socio-political situation.

For example, not only does the author describe in great detail the complicated events which followed the discovery of the Moabite stone, events which finally led to the tragic destruction of the monument, but adds a new dimension by setting the whole sad saga against the backdrop of the equally intricate international relations among Great Britain, France and Germany just prior to the Franco-Prussian war. The near frenzy which accompanied the desires of the various scholars to acquire this monument is explicable in terms of its outstanding importance. For the black basalt stela, discovered at the site of biblical Dibon, commemorates the successful attempt of Mesha, King of Moab in the ninth century BC, to liberate his country from Israelite domination. It was, therefore, one of the first and most spectacular examples of an ancient inscription corroborating a biblical narrative, for the same events, albeit with a changed nationalistic bias, are recorded in the Second Book of Kings (II 3: 4-27).

So too, Silberman helps us to understand that the series of really massive excavations conducted by the *Deutscher Palästina Verein* during the early years of this century, e.g. Sellin at Ta'anach, Schumacher at Megiddo, and Sellin and Watzinger at Jericho (Carl Watzinger was the co-director at Jericho, not Schumacher as stated by Silberman), were conceived as part of a deliberate German policy to support and strengthen the failing Ottoman Empire as a buffer against its fragmentation at the hands of the British, French and Russians.

Ultimately, however, this is a book of personalia and Silberman paints vivid, if not always flattering portraits of many of the giants of the subject. Charles Clermont-Ganneau, the brilliant French archaeologist and scholar, comes off worst, depicted as the sort of man to whom one would not tell the time of day for fear of it being denied and ridiculed in the *Times*. In contrast the author gives a sympathetic account of the rather pathetic antiquities dealer, Shapira, whose almost certainly genuine "Dead Sea Scroll" fell prey to the same Clermont-Ganneau, an experience which was to lead eventually to the poor man's suicide. Even some of the best forgotten exploits are enthusiastically remembered by Silberman, such as Seetzen's ill-prepared and disastrously executed survey of Eastern Palestine or Montague Parker's outrageous search for the treasure of Solomon beneath the Dome of the Rock in Jerusalem.

Frontispiece of C. Conder's *Tent Work in Palestine* (1878)

Much attention is rightly given to the outstanding and pioneering work of the Palestine Exploration Fund. It was this organization more than any other which changed the course of exploration in the Holy Land, removing it once and for all from the hands of the wealthy dilettante and firmly establishing it as an academic and scientific discipline. Today, Palestinian archaeology is the most sophisticated of all the branches of Near Eastern archaeology, with refined stratigraphic excavation techniques and pottery chronologies capable of differentiating between periods as little as 50 years apart. Silberman successfully shows how the groundwork for these achievements was laid: ironically perhaps, two of the great innovators, Sir Flinders Petrie and George Reisner, were "borrowed" from Egyptology.

Relevant and interesting illustrations have been chosen to accompany the text, and the map showing the location of the various late nineteenth and early twentieth century excavations is a most useful adjunct. Altogether, this is an excellent book which can be recommended to both specialist and non-specialist. For students of Near Eastern archaeology, it must be placed in the category of essential reading.

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Variations on the theme of cartography

Sarah Tyacke

Early Thematic Mapping in the History of Cartography. By Arthur H. Robinson. Pp.266. ISBN 0-226-72285-6. (University of Chicago Press: 1982.) $35, £24.50.

OPEN any atlas today and the chances are that many of the maps you see will be thematic, that is they show the distribution of some particular phenomenon stretched across a base map. The themes may range from the physical structure of the world to any aspect of human life, including abstract concepts; they are mapped by a number of graphic methods, immediately understandable by almost everyone, such as dots, flow lines, isolines and various types of tonal shading. These cartographical commonplaces were rarely, if at all, in evidence before 1700 and only took off in the first half of the nineteenth century. Neither the idea of thematic mapping nor the techniques for doing it were widely disseminated in intellectual and other educated circles of earlier times.

As the result of many years of research, A.H. Robinson has recovered the history of thematic mapping and vividly recounted its tender early years and its remarkable flowering under the impact of official and other statistical gathering from the end of the eighteenth century onwards. Unlike the general history of cartography, Robinson has few predecessors in this field and has provided *ab initio* the framework into which the maps gleaned from the works of statisticians, medical doctors, geologists, administrators and others may be slotted.

Although such maps are now very much the province of geographers and allied professionals, the earliest maps, so termed, were more usually drawn to illustrate or to analyse particular distributions or correlations between phenomena and the countries concerned by specialists in other fields; as such the maps are not to be found in atlases but are attached to official reports and professional papers, and illustrate a variety of subjects. For example, medical statistics on cholera were transformed graphically by Dr John Snow in 1855 to demonstrate the relationship between contaminated water supplies and the disease in London: it is these types of maps that Robinson describes and illustrates in the book.

Robinson has felt obliged to narrow his view of thematic mapping, however, to what he regards as the thematic map *sensu stricto*:

> ideally the thematic map has as its purpose the display of the overall structure or meaningful character of a distribution rather than simply showing where some things are.

In so doing — and additionally because he does not see some classes of thematic map as contributing to the development of graphic means of representation of data on maps — a number of types of thematic maps are not considered: for example, land use maps and other categories which may perhaps be called "special purpose" maps are not included. In the latter grouping might be mentioned such items as military plans, road maps and ecclesiastical maps. These types may or may not differ in graphic representation from that found on topographic maps but their function is primarily for a specific purpose and often illustrates a theme. In the case of ecclesiastical mapping a typical thematic example would be an atlas describing the numbers and distribution of properties owned by the Capuchin monks dated 1712. A key to the numbers of monasteries, priests, monks and lay brothers accompanies each map. The idea of representing the numbers by some manner of proportional symbolism is not present but the thematic element is quite obvious in this type of map.

Although it would indeed seem to be the case that the introduction of various thematic maps and symbols did not, as Robinson shows, occur before the end of the seventeenth century, it is perhaps unwise to assume that no one had previously conceived of the idea of depicting the structure of one phenomenon on a map. Certainly such an idea was not at all common but it is evident, even from some sixteenth century texts and maps which survive, that at least some people came close to the idea; for instance Queen Elizabeth I's minister William Cecil drew a map of the south-west of England and Wales showing the distribution of copper and lead mines in the 1570s. If one can see in this and similar attempts appreciation of some of the practical uses of such mapping, it must also be admitted that these maps show the qualitative location of phenomena rather than their quantitative distribution. It is quantitative mapping and its methods of representation which, as Robinson rightly points out, only emerge in the first half of the nineteenth century as a response to statistical studies.

The book is completed by 110 illustrations, many coloured, which are for the most part models of reproduction. In particular the selective use of legible detail rather than the reproduction of entire maps, which are so often indecipherable, is most welcome. Beyond this the great value of Robinson's work is that he has not only found a great many examples of thematic mapping, however defined, across a wide spectrum of professional endeavours, but has then produced a convincing and illuminating history which may be truly regarded as seminal. ☐

Sarah Tyacke is Assistant Keeper in the Map Library of the British Library, Great Russell Street, London.

Lamarck the Mythical Precursor
A Study of the Relations between Science and Ideology
by Madeleine Barthélemy-Madaule

Translated by Michael Shank, this book presents a highly readable account of Lamarck's theories and the debates they generated. He emerges as a bold and intellectually adventurous pioneer whose early work centred on meteorology and botany and who became the leading authority of his period on invertebrates The author does not attempt to rehabilitate Lamarck, but shows that his theories are still relevant to the debate on innate versus acquired characteristics. November 1982, £12 25

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October 1982, £10.50.

Nature's Second Kingdom
by Francois Delaporte

"[Delaporte's] project is to chart the concepts and practice of 18th century discourse on 'vegetality' . . He shows how these were related to questions about the nature of life and, most important, he argues that the epistemological standards of knowledge about plants was *animal* physiology." — *Annual of Science.* Translated by Arthur Goldhammer May 1982, £14.00

Genetic Alchemy
The Social History of the Recombinant DNA Controversy
by Sheldon Krimsky

This book offers a probing analysis of the setting for the rDNA debates, the postulated biohazards, the risk assessment process, the politics of science and regulation, and the means through which biologists protected their discipline October 1982, £17.50

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Changing visions of an ancient society

Arthur A. Demarest

Ancient Maya Civilization. By Norman Hammond. Pp.337. Hbk ISBN 0-521-24017-4; pbk ISBN 0-521-28399-X. (Cambridge University Press/Rutgers University Press: 1982.) Hbk £22.50, $27.50; pbk £7.95, $12.95.

A MILLENNIUM ago the dense Peten jungle of Central America swallowed up many of the sprawling cities of the ancient Mayas, entombing their lofty temples, elegant palaces and graceful sculptures beneath a blanket of vegetation. During the past century and a half archaeologists, scholars and adventurers have spent much of their lives trying to unravel the mysteries of this lost civilization. *Ancient Maya Civilization* by Norman Hammond of Rutgers University is the most recent presentation of their findings and of our understanding of ancient Maya history and culture.

The Maya-speaking Indians of eastern Mexico and northern Central America have a heritage that can now be traced back for over 3,000 years. It was, however, the Classic Period (AD250 to 900) that witnessed the florescence of Maya culture — leaving a legacy of hundreds of archaeological sites in the jungles of northern Guatemala, Belize and the Yucatan peninsula. During this golden age, great centres such as Tikal, Palenque and Uaxactun flourished in an environment which appears utterly inhospitable to European and North American eyes. The hallmarks of this period are its fine masonry architecture, monuments sculptured in delicate bas-relief, raised stone roads and polychrome-painted ceramics whose beauty rivals the vase painting of Classical Greece. The sophistication of the Classic Period of Maya culture can be seen in its fully developed writing system, only partially deciphered, which records calendrical calculations and astronomical knowledge of astonishing accuracy — all based solely upon relentless non-telescopic observation and recording. Despite these cultural achievements, the great jungle cities of the southern zone of Maya culture were abandoned some time about AD900. Archaeologists have suggested various causes for the fall of the southern lowland centres including invasion, disease, ecological mismanagement, peasant revolt or complex combinations of such factors. Yet no consensus has been reached and this collapse remains a great mystery.

Hammond's *Ancient Maya Civilization* could not be more timely, since it comes fast upon the heels of a revolution in Maya archaeology. For half a century, scholars have understood the general nature of the Maya intellectual achievements. These have been beautifully described in the classic works of great Mayanists such as J. Eric S. Thompson and Sylvanus Morley. However, we can now see that these scholars misunderstood many fundamental aspects of Maya culture. The Maya were characterized as isolated, peaceful, theocratic societies supported by small populations of peasants using simple slash-and-burn agriculture and governed by priests concerned only with the worship of time and with astrological lore. Hammond's book incorporates the most recent research on settlement, agriculture and hieroglyphics, which shows Classic Maya society to have been far more dynamic than previously believed. Breakthroughs in hieroglyphic interpretation have revealed that the Classic Maya political landscape consisted of petty lords battling for control of regional city-states. Meanwhile, large-scale archaeological surveys have uncovered complex agricultural systems — raised fields, canals, terracing and other means of water control — which supported dense (proto-urban) populations.

Hammond artfully combines the knowledge gained by the early scholars with the most recent interpretations. First, he draws in the reader unfamiliar with the subject with dramatic, at times romantic, chapters on nineteenth-century explorations of the Maya ruins and twentieth-century archaeological projects. The following, more substantive, chapters on Maya ecology and cultural history are rendered accessible by numerous illustrations, maps and charts. The tone then shifts in thematic chapters on subsistence, politics and trade, in which Hammond presents the evidence for the new, more realistic, characterization of ancient Maya society. Yet in the subsequent accounts of Maya architecture, art and religion, he draws heavily upon the works of the early Mayanists with an undisguised admiration. Hammond also maintains a balance in his discussion of still controversial topics, such as specific interpretations of Maya dynastic history, foreign influences on the Maya and the causes of the Classic Maya collapse. In short, the author is surprisingly successful in integrating old and new conceptions of the ancient Maya.

The book, however, is less successful in dealing with the regional diversity of the civilization. Hammond himself is an active field archaeologist, excavating in Belize, and his text reflects a regional bias towards the southern, lowland manifestation of Maya culture. In the chapter reviewing the rise and fall of Maya civilization, he gives only the most superficial treatment of the development of the great centres in Yucatan, the Guatemalan highlands in the south, and the south-eastern frontier in El Salvador and Honduras. In the thematic chapters, Hammond similarly draws most of his examples from the same handful of sites in Belize and the southern lowlands. Students and laymen reading Hammond's outline of Maya history also should be made aware that his very early datings for the beginnings and development of complex village societies in the lowlands (which I personally accept) are still being hotly debated.

These criticisms notwithstanding, the book is an excellent introduction to the ancient Maya. Indeed, even the author's research biases, when combined with his crisp journalistic style, help to convey the fluid nature of Maya archaeology in which interpretations are undergoing constant revision as each excavation unearths new contradictions and fresh controversies. Hammond's book achieves a unique fusion of a reverence for the early archaeologists and explorers with an insistence on the most current interpretations. It is a perspective which gives the reader a new vision of both this lost civilization and the volatile discipline that is rediscovering it.

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A ring of irony

J.W. Cornforth

The Enchanted Ring: The Untold Story of Penicillin. By John C. Sheehan. Pp.224. ISBN 0-262-19204-7. (MIT Press: 1982.) $15, £10.50.

IN 1941, when Howard Florey's team at Oxford had demonstrated the value of penicillin for treating bacterial infections, it became imperative to find better ways of producing the drug in quantity. A huge cooperative effort by 39 industrial, university and government laboratories in the United Kingdom and United States explored not only the existing method of fermentation, but also the chemistry of penicillin in the hope of producing it by synthesis if fermentation techniques could not be improved. Research on the fermentation process was brilliantly successful, and the problem of producing penicillin was solved. In contrast, although the chemical effort succeeded in finding out the structure of penicillin it failed to find an efficient method of synthesis.

As the urgency of the problem faded, chemists engaged on the synthesis of penicillin turned to other work. The most notable exception was John Sheehan, of Max Tishler's team at the Merck laboratories. Leaving Merck for the Massachusetts Institute of Technology after the war, Sheehan sought a rational synthesis of penicillin and in the end he found one. His book is partly an account of his personal involvement with the penicillin problem and partly an anecdotal story of the drug's discovery and development. It is difficult to picture the readership at which Professor Sheehan is aiming; but the book will certainly interest, and probably irritate, all those who have worked on penicillin.

Sheehan's account of the early days of penicillin reads largely as an attempt to magnify Fleming's contribution at the expense of Florey's and Chain's. He throws no new light on this old controversy. More novel is his account of the organization behind the research and production effort in the United States in 1941–1944. He draws here on minutes of the US Committee on Medical Research, and other official papers. He has recorded on tape the reminiscences of some principal participants, notably Karl Folkers, and he reproduces extracts from these, along with an account from Hamao Umezawa of a belated Japanese effort to produce penicillin in 1944–1945. The absence of chronological order makes difficulties for the reader, especially in a book so obsessed by priority and credit. The difficulty persists throughout.

The part of the book dealing with chemistry is in many ways the most disappointing. Chemists are likely to be put off by the unexpectedly poor appearance and layout of the diagrams, and by oversimplification that sometimes sacrifices truth, whilst the non-chemical reader may well be confused and misled. This is particularly true of the chapter where one would expect it least — the description of Sheehan's own work. A brief account, based less on the book than on the scientific literature, seems indicated here to make clear Sheehan's contribution.

The molecule of penicillin is relatively small and simple, and the difficulty of synthesis arises mainly from the presence of several reactive groupings that cannot easily be manipulated without mutual interference. It was already recognized by the end of 1943 that pencillin is an anhydride of penicilloic acid, a substance easily made from penicillin and easily synthesized. A decision between two possible structures for this anhydride was eventually made in favour of the β-lactam structure, but it was found to be impossible to produce this from penicilloic acid or its simple derivatives. That was the problem that Sheehan solved. His earlier successes were obtained by making inert analogues of penicilloic acid that enabled him to use a quite vigorous reagent — thionyl chloride — to close the strained β-lactam ring. The breakthrough came when he discovered that carbodiimides could be used to form amides in mild conditions from carboxylic acids and amines; a most valuable peptide synthesis arose from this finding. The cyclization of penicilloic acid to penicillin is a special type of internal peptide synthesis, and when the carbodiimide method was applied to a suitable penicilloic acid a natural penicillin was formed in low yield. This work was submitted for publication in February 1957.

Sheehan's method for synthesizing penicilloic acids proceeded by way of an analogue lacking the acyl side-chain characteristic of penicillins. He tried the long shot of cyclizing this compound and obtained a crude mixture containing a very small proportion of racemic 6-aminopenicillanic acid (penicillin without the acyl side-chain). When this mixture was treated with an appropriate acyl chloride, formation of a penicillin could be shown to have taken place. Sheehan did not publish this experiment but he applied for a patent on it, in March 1957. In 1959, he modified the process by using a trityl group to protect the reactive amino group before cyclization; he then obtained much better yields in closure of the β-lactam ring, though the yields from subsequent removal of protecting groups were poor. This procedure gave him fully synthetic 6-aminopenicillanic acid.

In 1957, workers at the Beecham Laboratories isolated 6-aminopenicillanic acid in pure form by fermentation. This completely independent discovery was the basis for an enormous development of "semi-synthetic" penicillins carrying "unnatural" acyl side-chains. That whole development rests on aminopenicillanic acid derived from fermentations and no penicillin has been produced in commercial quantities by total synthesis. Nevertheless, Sheehan's commercially valueless procedure enabled him to gain, after a protracted legal battle, priority of invention. The last chapter in his book describes that battle.

Sheehan's achievement in making a total synthesis of penicillin was a splendid piece of work. His book, in contrast, lends its own sub-title — *The Untold Story of Penicillin* — a ring of irony. ☐

Sir John Cornforth is Royal Society Research Professor at the University of Sussex. He is a part author of The Chemistry of Penicillin *(Princeton University Press, 1949).*

Elks, Whelks, and their Ilk

The monarchs of the Irish bogs
Succumbed to neither men nor dogs
But (most ecologists agree)
To calcium deficiency.
They scoured the base-deficient peat
For antlers and old shells to eat
And, finding all too few, they died
Around the Celtic countryside.

. . .

Then mourn the passing of the elks,
But note the wisdom of the whelks
That roam the shore — their
native heath
With silver-indurated teeth
And bore to death their mollusc friends,
Who come to sad, unsuccoured ends.
Without the need for gins and limes,
The whelks survive to modern times.

. . .

Thus ungulate and gastropod,
And all that live by sea or sod,
Are doomed to be — or not to be
By biogeochemistry.

Ralph Lewin

Children of clay?

N.W. Pirie

Genetic Takeover and the Mineral Origins of Life. By A.G. Cairns-Smith. Pp.477. ISBN 0-521-23312-7. (Cambridge University Press: 1982.) £15, $29.50.

Eminent physicists have written so much cocksure balderdash about the nature and origins of life, that it is reasonable to be apprehensive about a contribution to the subject from a chemist. We have become accustomed to the naive assumption that, because life is now dominated by the behaviour of proteins and nucleic acids, a study of the conditions necessary for their probiotic synthesis and interaction is relevant. (If that assumption were valid, the right approach to the history of the use of fire would be to study the mechanism of a cigarette lighter, and a study of the composition of paper and ink would illuminate the history of writing.) Cairns-Smith argues instead that proteins and nucleic acids were successful late arrivals on the biological scene. This is an unpopular, but not wholly novel, point of view — a few of us have been adumbrating it for many years. What is novel is that he suggests a concrete alternative hypothesis, almost literally concrete because he gives silicates a fundamental role.

The argument is that clay minerals are not simple rock fragments, but are recrystallized structures with a final form which depends on the conditions of crystallization, the extent to which supersaturated solutions are seeded and the transmission of accidental defects. The last point will be the most controversial. An evolving quasi-biological system needs a genotype and phenotype: this is the old distinction between form and substance. In biology, contrary to Aristotle's dictum, it is the form, the genotype, that lasts and not the substance.

About a quarter of the book is occupied by a description of the immense variety of clay minerals, the distortions and defects in them, and their behaviour in the presence of other substances. The photomicrograms and electronmicrograms show complicated folded laths and other structures, colourfully described as "micro-origami", "card houses" and "a self-assembling maze". Even without the detailed and plausible argument which is presented here, the structures shown in these illustrations support the idea that, if prevital evolution happens anywhere, this is a reasonable group of substances within which to search for it. The old analogy between life and crystallization, which was discussed by Kant, Reil, Troland and others, may now legitimately be elevated from the status of analogy to that of hypothesis. Nevertheless, it may be argued that the mechanism suggested — for example a particular type of clay crystal perpetuating its ability to thrive in crevices in sandstone by shedding "seeds" into neighbouring crevices — is rather remotely connected with what we normally regard as life. That objection applies with equal force to the organic polymerization picture which has recently become conventional. Neither picture fills the gap between the never living and the nearly living in a wholly satisfactory manner. But it is pleasant to have a change of outlook.

Cairns-Smith chooses clay minerals as the vehicle for biopoesis because of their versatility and indubitable abundance on the probiotic Earth. However, evidence from meteorites, and from *in vitro* experiments going back to the time of Berzelius and Berthelot, make it equally certain that tars would also be present. Tars are the main product of the tediously repetitious experiments in which gas mixtures, made up according to constantly changing opinions about the composition of the probiotic atmosphere, are now exposed to various types of energetic insult. Tars are mentioned here several times. They are invariably disparaged as inert end-products or as "tarry chaos". I have been arguing for many years that sunlit tar is as likely a site as any other for an early stage of biopoesis, but after reading this book I would include clay minerals in the notional mixture and, following Goldschmidt, some minerals containing the transition elements. Everyone to his taste. Cairns-Smith is a clay enthusiast: he enjoys giving clay a role more dynamic than mere adsorption and sequestration.

There is no suggestion here that clay filled this dynamic role in the hypothetical eobiont any better than protein would have done. On the contrary, there is the explicit statement: "Anything clay could do organic molecules would eventually come to do better". To quote again, the discussion is said to be about: "What was biochemistry like when protein was an optional extra?". Several passages emphasize the point that eobionts need not have been small. The idea that life started small presumably stems from a fallacious conflation of early life with viruses. Being obligate parasites, viruses have obviously nothing to do with the case: it is not even useful to classify them as organisms. It is only when competition with other organisms puts a premium on rapid reproduction that smallness becomes advantageous. In the beginning there was no competition. As Cairns-Smith puts it:

> The microscopic size of the modern bacterium should be seen as one of its most notable achievements rather than any sign of evolutionary infancy. The bacterium is a highly concentrated package of ingenuity.

If minerals have the potentialities attributed to them here, it should be possible to demonstrate processes comparable to evolution taking place among them now. This point is discussed briefly with references to some as yet unconfirmed claims that such processes occur in various precipitates. It is not enough to show that there are progressive changes in these precipitates; to be convincing it should also be demonstrated that the change is hastened when a fresh precipitate is inoculated with a "seed". The absence of evidence for this does not invalidate the hypothesis. About a gillion (10^9) years seem to have elapsed between the time when physical conditions on Earth became comparable to those with which we are familiar, and the formation of the first organisms. Relevant *in vitro* processes need not therefore be rapid.

Evidence that the first organisms were inorganic could also come from an appraisal of the present-day biological role of minerals. Some organisms show great enterprise in exploiting specialized properties such as the magnetism and hardness of Fe_3O_4 and the density of $BaSO_4$. It has been suggested that the five-fold symmetry of most echinoderms is imposed by the 75° angle between the faces of calcite crystals, and it may be legitimate to wonder whether the greater intracellular importance of K rather than Na is a relic of the behaviour of these ions in clays. On the whole however, such typical mineral elements as Al and Si seem to have been steadily eliminated. It is primitive plants that make most use of Al, and primitive animals of Si — though there are still substantial amounts, with obscure functions, in the human body. Clearly, evidence for a mineral background to biology is tenuous: the postulated takeover is nearly complete. We are, on the whole, reasonably satisfied with the result. Sir Walter Scott's words are apposite and partly justify the title of this review: "Well hast thou done, frail child of clay!".

Recent articles and letters to the Editor of *Nature* have shown that there is disagreement about the principles that operate and have operated in evolution. The theme often crops up in this book. At a time when we are still arguing about the survival value of many queer features in living organisms, some of the statements made have a rather over-confident tone. It will be interesting to see what comments on that aspect of Cairns-Smith's thesis are made by reviewers with a primary interest in evolution rather than chemistry. Cairns-Smith is commendably tentative, he recognizes that a crystalline gene might not work and says that: ". . . when we are near to the answer we will find many similar answers, and so we will not be able to say exactly how life must have started on Earth". He writes clearly with many neat phrases and some humour. The result is an excellent book — the most plausible and sensible that I have seen on the subject. □

N. W. Pirie was formerly Head of the Biochemistry Department at Rothamsted Experimental Station, Hertfordshire. He has been writing articles on the nature and origin of life since 1937.

How to get the wind up in the future

F.J.P. Clarke

Wind Energy for the Eighties: A Review by Members of the British Wind Association. Pp.372. ISBN 0-906048-73-7. (Peter Peregrinus: 1982.) £19.50, $49.50.

Is wind energy a credible source of electricity for the world, and in particular for the UK? *Wind Energy in the Eighties*, a status review written by experts from the British Wind Energy Association, gives a clear answer: wind energy could readily contribute 20 per cent of the UK's electricity supplies and it could be well on the way to that by the year 2000. How valid is that claim?

Although detailed resource surveys have still to be carried out both on land and offshore, the book explains why resource size is unlikely to be a limitation. Because the wind is an unreliable source of power, our electricity system is limited in what it can accept to perhaps 20 per cent of total demand. But that much at least the Electricity Boards agree can be accepted; and since the feasible resource base is likely to be much greater than that, resource size is unlikely to be limiting — provided, that is, the economics are right.

There are two aspects to this latter question, the value of wind power and its cost. Because of the unreliability of wind power, its value must be judged primarily in terms of the conventional fuels it displaces when the wind is blowing, which depends on the plant mix — how much coal or oil will be saved? And that depends on how many wind machines are in the system; the more wind machines there are, the smaller is the value of the next machine to come on stream because the highest running-cost conventional plant will always be displaced first. Modelling such system economics is difficult; present models may be inadequate, and the fact that one is concerned with a plant mix well into the future adds to the difficulties. Nevertheless estimates can be made and are given in the book, assumptions being clearly stated.

The second aspect of the economics is the cost of the wind machines themselves and their associated plant. Here, overseas estimates tend to come out lower than those made in the UK. While this may in part be the problem of relating US dollars or Swedish kroner to British pounds, the book does point up differences in technical assumptions that also contribute.

From all these facts, speculations and assumptions the balanced conclusions emerge that wind machines can be integrated into the electricity grid in substantial numbers, and that in some cases it should be economically worthwhile to do just that.

If these conclusions seem modest against the claims sometimes made for wind power, it should be remembered that they are formulated by professional scientists and engineers who are as aware of the uncertainties as of the very real promise of the technology. More knowledge is needed to lower costs and encourage adoption of the technology, especially offshore.

One of the important concepts behind the possibility of lowering costs is the compliant structure. The design approach in the UK has so far been based upon structural stiffness to avoid resonance and vibration which are notoriously difficult to predict, and which can be so damaging. Such thinking was also behind the earlier US designs. However, subsequent US, Swedish and German designs have gone for a more compliant structure. This involves a much better understanding of the dynamics of the whole structure so that lighter, smaller and less-expensive components can be used. The dynamic forces can then be absorbed with compliance rather than with stiffness. As is admitted in the book, this approach is rapidly gaining favour as an important way to reduce costs, though the extent to which the concept can be applied in the highest wind regimes in the UK has still to be determined. However, as reflected by the cursory treatment, the UK is lagging in this area.

Wind Energy Group

The shape of wind machines to come — an artist's impression of the wind turbine to be built on Burgar Hill, Orkney, by the Wind Energy Group. Overall the structure is 75m in height, taller than Nelson's Column in London.

Two basic types of machine are being developed industrially in the UK and both receive good coverage together with their overseas counterparts. The horizontal axis machine had the earlier start, and this has been followed by the vertical axis concept. The rotor blades of horizontal axis machines are subject to reversing gravitational loadings as they turn, thus making them the components most at risk from fatigue failure. The vertical axis machines avoid this and have the additional advantage of being omnidirectional; however they would be more prone to other problems which need investigation.

At megawatts size either machine would look large alongside the traditional windmill and could reach higher than the largest electricity pylons, thus raising the problem of visual amenity. Also, there have been reports from the United States of television interference, windows rattling and other environmental impacts; but so far they seem to be rather isolated events. On this type of issue, as on techno-economic issues, real answers will come only when large machines are up and working in the UK.

Such possible environmental problems make it attractive to place machines in shallow waters offshore. This would cost more, but, it is claimed, expense may be outweighed by the higher wind speeds and the environmental freedom to build bigger structures. Whatever these pros and cons, different practical and institutional advantages are also covered in the book. Wind machines of 1–10MW provide a modular power source that can be added to the system quickly as needed, without all the well-known problems of massive construction projects. In addition, it is pointed out that such machines could provide the beginnings of decentralized power supplies — a possibility that could attract support across political boundaries.

Wind Energy for the Eighties brings together professional views from the academic world, from industry both public and private, from research institutions and from countries overseas. Apart from its value as a status report, the book sets out simply and clearly the background theory to many of the topics. Students and those new to wind energy will find it as good a source book as those already in the field. It is a pleasure to welcome it.

F.J.P. Clarke was Chairman of the National Wind Energy Steering Committee until 1981. He is now Director of Strategic Studies at the United Kingdom Atomic Energy Authority.

Cornucopian faith

Eric Ashby

Catastrophe or Cornucopia: The Environment, Politics and The Future. By Stephen Cotgrove. Pp.154. Hbk ISBN 0-471-10079-X; pbk ISBN 0-471-10166-4. (Wiley: 1982.) Hbk £16, $35; pbk £6.95, $15.30.

OVERHEARD in a pub, two women talking: "She was as stubborn as a mule. But I was just as firm". This is the theme of Professor Cotgrove's book: the controversy between those he calls catastrophists and those he calls cornucopians, those who predict a collapse of industrial society and those who believe that threats to our survival will continue to be kept at bay by the very products of that society. Each side so sure it is right and so ready to dismiss the other side as irrational. Each side having access to the same set of facts but interpreting them so differently.

Professor Cotgrove is a sociologist who believes in using quantified data prepared from questionnaires for an analysis of what a layman would call "public opinion" on environmental issues. His aim is to discover what sort of people are catastrophists, and what sort cornucopians. He wisely discards these picturesque (and in my view misleading) labels for more specific categories: environmentalists (a sample of 576 persons from the Conservation Society and the Friends of the Earth), industrialists (a sample of 400 from *Business Who's Who* and *Who's Who of British Engineers*), trade union officials (399 names from the *Trade Union Handbook*), conservationists (500 names from the World Wildlife Fund, UK Branch), and, to represent the public, 1018 names drawn from the electoral registers of Bath, Oldham and Swindon. These people were asked to respond to a battery of questions about their attitudes to the environment, to various other social issues, to the effectiveness of the present political system, to the morality or otherwise of the imperative of economic growth. With this background of information Professor Cotgrove pursues his aim, concentrating largely on environmentalists on the one hand and industrialists on the other.

The initial results cannot be described as surprising: 92.4 per cent of environmentalists consider environmental problems to be "extremely" or "very" serious; only 45.9 per cent of industrialists share this view. Each category of the public behaves predictably. The environmentalists were selected from a sub-group which has deliberately carried its campaign into politics; belief is often hardened into ideology. Conservationists are milder people, less politicized, inclined to give a higher priority to law and order and to the economy than they do to environmental issues (except the conservation of the species to which they are attached: birds, or whales, or redwoods). Industrialists naturally put their faith in the creation of wealth and tend to underplay anything that threatens that faith. Trade union officials find themselves, as one would expect, sitting on the environmental fence. Of course they want improved social conditions, less pollution, public finance for environmental tasks, but when it comes to jobs versus the environment one doesn't need Professor Cotgrove's statistics to provide an answer.

After this somewhat pedestrian start the book gathers pace. Environmentalists and industrialists emerge as the personifications of two opposing life-styles which Professor Cotgrove (unwisely, I think, for it begs the $64 question in the book) calls "paradigms". The typical environmentalist is in early middle age, well educated, engaged in what industrialists would call a non-productive occupation (as teacher, social worker, academic, doctor), left wing in politics (unlike conservationists, who are commonly right wing), hostile to the current enthusiasm for economic individualism and commitment to economic growth. The typical industrialist in the sample is over 40, also well educated, engaged in production or marketing of commodities, right wing in politics and living — except in his home life: the book makes that distinction — by the strenuous survival standards of the market place.

In this crude classification some truth, of course, is lost. If environmentalists and industrialists were victims of their respective paradigms there would be no cross-fertilization of ideas between them. It's not as bad as that. It was the industrialist who successfully flooded Latin America with mass-produced automobiles, Aurelio Peccei, who founded the Club of Rome and has become the high priest of environmentalists. So not every reader will agree with Professor Cotgrove when he says:

> Industrialists and environmentalists . . . inhabit different worlds. From where they each stand, the world looks different. What is rational and reasonable from one perspective is irrational from another.

It is a caricature, but the merit of caricatures is that they do carry some truth. It is not necessary to swallow the whole of Professor Cotgrove's thesis in order to get from him some useful, if uncomfortable, ideas. The idea that strikes me as most important is the suggestion that the issues environmentalists get so worked up about — acid rain, nuclear waste, lead in petrol, draining of wetlands — are symbols of a much deeper rejection of the social order that finds it fitting to exploit the environment in these ways. Accordingly, though the industrialist and the environmentalist might agree on the desirability of taking lead out of petrol, they would act for totally different reasons: the one, out of expediency, the other as a step toward ridding the air of a moral, as well as a material, impurity. The book quotes the well known work by Mary Douglas on the anthropologist's concept of pollution; it might, even more appropriately, have quoted the traditional Middle English meaning of the word: "to render ceremonially or morally impure, desecrate, destroy the sanctity of. . .".

For this is what Professor Cotgrove is driving at: the idea that environmentalists are not out just to tidy up land, air and water (to do no more than that would be rejected as "cosmetic" treatment). They are out to overthrow the lifestyle that has, in their view, desecrated the countryside and de-humanized cities, and to put in its place what Illich calls "conviviality". A few years ago this sort of protest would have been academic in the pejorative meaning of the word. But a change has occurred that makes this protest potentially significant: it is the undeniable loss of confidence in the capacity of our political system to cope with our social and economic problems, a "decline in political legitimacy". To come back to Professor Cotgrove's statistics: it is not surprising that 74 per cent of environmentalists have little trust in political parties; it is surprising (at any rate, it ought to be) that 68 per cent of industrialists, too, have "little trust". And even more surprising that, when asked whether they support direct (extra-parliamentary) action to influence government decisions on environmental issues, there is still support from 23 per cent of the industrialists who responded to the questionnaire. The framework of consensus within which protest groups could easily be tolerated is showing ominous signs of weakness. Professor Cotgrove suggests (as have, of course, many others before him) that the change from industrial to post-industrial society may not be a smooth one. Indeed, if he is right to use the word "paradigm" for the ruling lifestyle and the challenge being made to it, then the two paradigms cannot co-exist (that is why I don't think "paradigm" is the best word to use). And the question becomes not "whether?" but "how?" the transition will take place.

It is at this point, at the end of the book, that the reader may wish Professor Cotgrove had carried him a bit farther. We have to be content with I'd call the Heilbroner formula: get your house in order by constitutional means, or you'll find the constitution swept away and replaced by Leviathan. The urgent need is for reflection on the kind of political institutions which might evolve, from what we have to what we need. In his book *Ecology and the Politics of Scarcity* (which Professor Cotgrove does not refer to) William Ophuls has made some suggestions which could be the prolegomenon to an environmental ethic. Sociologists have an important part to play in finding out how such an ethic could be disseminated among a people still living (as Professor Cotgrove puts it) under a "Cornucopian faith". □

Eric Ashby is a Fellow of Clare College, Cambridge. He is joint author with Mary Anderson of The Politics of Clean Air, *published by the Clarendon Press in 1981.*

Keeping nuclear weapons inside the club

Stephen M. Meyer

Controlling the Bomb: Nuclear Proliferation in the 1980s. By Lewis A. Dunn. Pp.209. Hbk ISBN 0-300-02820-2; pbk ISBN 0-300-02821-0. (Yale University Press: 1982.) Hbk $21, £16; pbk $6.95, £5.45.

Nineteen eighty two has been a fairly active year for watchers of international conflict. Britain and Argentina went to war over the Falkland Islands. The Iran–Iraq War flared to new levels of ferocity, culminating in the largest tank battles since the Second World War. Meanwhile, Israel engaged PLO and Syrian forces in Lebanon.

It is worth pondering how each of these conflicts might have evolved had they taken place in 1992, when many of the protagonists might have added nuclear weapons to their arsenals. What might have been the result of the detonation of an Argentine nuclear warhead in the midst of the British naval task force? What would have happened if the Iraqis, Iranians or both had nuclear bombs for their tactical aircraft? And imagine the effects of sudden demonstration by the PLO that it had acquired a nuclear weapon (by theft, purchase or gift). In fact, none of these is implausible, and unless we wish to see such items in future news headlines we must begin to take a more serious and systematic approach to coping with the problem of nuclear weapons proliferation.

It is in this context that Lewis Dunn's *Controlling the Bomb* makes several timely contributions to our understanding of the spread of nuclear weapons, the threats posed by further proliferation and the options for slowing its pace and scope. In what is, perhaps, the most important aspect of this book, Dunn discards two pernicious notions currently much in vogue: that nuclear proliferation is essentially a technological phenomenon, and that further proliferation may have benign — if not beneficial — effects.

The first notion has dominated the literature since the beginning of the 1970s. Nuclear weapons proliferation was seen as a genuine technological imperative, a deterministic outgrowth of the presence of nuclear power plants. Accordingly, the eradication of nuclear power technology (and associated plutonium stocks) was prescribed as the most effective approach to minimizing, if not eliminating, that threat. In reviewing events of the past decades, Dunn exposes the fundamental fallacy of this view. While technical difficulties have indeed been a factor in some cases of nuclear restraint, decisions to pursue — or abstain from — the acquisition of nuclear weapons have been driven by politico-military considerations. Efforts by Israel, South Africa, Libya, Iraq and Pakistan to acquire nuclear weapons cannot be explained by the corrupting influences of indigenous nuclear power generation because the former preceded the latter. Conversely, the failure of West Germany, Japan, Sweden, Switzerland, Canada, Belgium, South Korea and some two dozen other countries to produce nuclear weaponry cannot be accounted for by a lack of expertise. In either case, the total eradication of nuclear power technology would not affect these countries' long-term capability of putting together a nuclear weapons programme using indigenous resources.

As Dunn implies later, technical problems do play an important role in non-proliferation efforts simply by buying time. However, the effectiveness of technological constraints is undergoing continual erosion. Dunn points to the sources of trouble. In their rush to increase export revenues and markets, the primary supplier states have been backsliding on controls over sales of sensitive nuclear know-how and products. Grey and black marketeering have added to the flow of nuclear goods. Compounding the problem is the rise of third-tier nuclear suppliers — India and Argentina, for example. In the end the ability to produce nuclear weapons is inherent in the scientific, technological and industrial growth that is part of economic development.

While he does not see an inexorable drift towards the further spread of nuclear weapons, Dunn acknowledges that there are likely to be a number of instances where the confluence of technical expertise and political will produces new members of the nuclear club. Is there any reason to interfere, to try to alter the pace and scope of proliferation? Here Dunn confronts the second misconception. The presence of nuclear weapons, it is argued, can bring peace and stability to conflict-prone areas, such as the Middle East, by establishing regional deterrent systems similar to that which has prevented a US–USSR war for almost 40 years. Just as the porcupine can walk safely through the forest, so too can the small nuclear power dwell among a world of hostile countries. In explicitly rejecting such bizarre formulations, Dunn points to a host of factors that invalidate these analogies, while suggesting considerable threats ahead: the geographical proximity and political instability of those countries which may come to possess the bomb, superpower relations with such nations, and terrorist activities.

Recognizing the clear threat posed by the wider possession of nuclear weapons, the second half of *Controlling the Bomb* outlines a range of alternatives for a US anti-proliferation strategy. The first set of measures is aimed at checking the pace and scope of further proliferation. Dunn argues that — in order to buy time — technical barriers should be increased so as to make entry into the nuclear club as difficult as possible. Dunn notes that pilot-scale and research facilities can pose a greater threat of proliferation than full-scale commercial nuclear power facilities. Also, the United States should continue to limit the number of countries engaged independently in fuel reprocessing, while re-establishing itself as a reliable supplier of nuclear fuel. Multinational nuclear research and energy programmes (for instance to deal with international plutonium storage) should replace national projects, thereby giving developing countries access to advanced nuclear technology but without the risks associated with independent national programmes. Closing of technology export loopholes is necessary as well.

In order to affect relevant incentives and disincentives, Dunn also proposes a US strategy of political, economic, military and diplomatic sanctions. The objective is to impose such large drawbacks that they overwhelm any existing urge to acquire nuclear weapons. As Dunn emphasizes, this can only be accomplished through case-by-case analysis and direct intervention, not by some global or universal non-proliferation policy. In this respect, he notes — but, unfortunately, does not pursue in great detail — the fact that day-to-day US foreign policy does have an important effect on the balance of proliferation incentives and disincentives; for example the pursuit of other, frequently more important, foreign policy objectives may conflict with non-proliferation interests as often as they reinforce such interests.

A second set of measures is concerned with "limiting damage" in face of further proliferation. Dunn argues that there may be "natural" stopping points in the development of nuclear weapons — so-called proliferation firebreaks — behind which nations could be held by the threat of sanctions and other forms of diplomatic intervention. Where the drive towards the acquisition of a nuclear arsenal could not be prevented completely, emphasis could be placed on retarding the futher evolution of the budding, but still small, nuclear force. Some countries might be contained at the convert (untested weapons) stage (e.g. Israel); some at the PNE (peaceful nuclear explosive) stage (e.g. India); some at the small stockpile stage; and so on.

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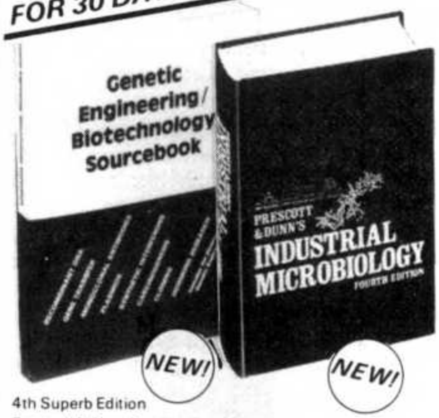

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Lastly, the task of mitigating the consequences of nuclear proliferation is addressed. Dunn considers three thresholds to contend with: after nuclear weapons production is first detected; after a first nuclear test; and after nuclear use in warfare. An assortment of possible US responses is suggested. Technical assistance might be offered after the fact to reduce the dangers of unauthorized or accidental use. Security guarantees could be given to non-nuclear weapons states likely to be affected by the newly born nuclear power. Adjustments to alliance relationships may be required. And finally, consideration must be given to military intervention against a budding nuclear force that poses a clear threat to peace, or against one that comes under the control of non-governmental forces.

Dunn concedes that many of the policy options in each of these three categories of control conflict with one another. As a result, the possibilities for developing a coherent US non-proliferation strategy are quite small. Thus, the reader should not be surprised to find no single blueprint for ensuring a world in which nuclear-weapons are no more widely available than they now are. Controlling the bomb will require careful attention to decision-making in individual countries, and to the development of an approach to the problem that has the flexibility and versatility to tailor responses to the case at hand. Dunn deserves much credit for getting our thinking back on the right track. □

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Bytes and pieces from an expert hand

Margaret A. Boden

Machine Intelligence and Related Topics: An Information Scientist's Weekend Book. By Donald Michie. Pp.316. ISBN 0-677-05560-9. (Gordon & Breach: 1982.) $55.

DONALD Michie has played a crucial role in the development of artificial intelligence (AI) in Great Britain. He was a moving force behind the first British AI department, set up nearly 20 years ago in Edinburgh and recognized as of world-class stature. His commitment and enthusiasm derives partly from his friendship as a young man with Alan Turing (a colleague at Bletchley during the Second World War), on whose work our understanding of computation is grounded. The experience at Bletchley was influential too in its emphasis on the applications and social context of science, and on the value of interdisciplinary cooperation in scientific research.

Michie's intellectual pedigree commands attention, and not surprisingly there are many nuggets of interest in his book. But this collection of papers is, overall, disappointing. It is an uneasy mixture, ranging from ephemeral two-page journalistic pieces, through accounts of AI aimed at the general reader, to technical discussions of chess programs and probability theory. It includes personal memoirs of Turing, advice to eminent scientists in danger of being seduced into administration and circuit-lecturing, and impassioned criticism of the Science Research Council's Lighthill Report — which seriously hindered the progress of AI in Britain a decade ago and from which the field has not yet recovered.

The examples of machine intelligence on which Michie concentrates are expert systems and chess programs. Expert systems are codifications of knowledge and inference about specific topics, such as geology or the diagnosis of infectious meningitis. The general interest of these is obvious, and his description of a variety of such knowledge-based systems (some of which are already in professional or commerical use) is lucid and informative. Chess programs might appear to be mere toys, but Michie argues that chess is especially well-suited to the experimental development of AI. It is a complex but relatively well-understood and clearly defined domain, and it demands research into techniques of heuristic search, pattern-recognition and inductive learning. AI work in other areas — such as language-understanding — is mentioned, but does not receive the detailed attention given to chess and expert systems.

Each paper in this collection was appropriate, even useful, in its original context. Michie is technically sophisticated, lucid in expression, historically sensitive and imaginative with respect to the future potential of AI. Few readers will find nothing of interest here. But it would be a strange person — even if he was that odd beast, an information-scientist — who would devote much of a weekend to reading this book. Despite Michie's advice that eminent scientists should spend their time doing innovative research rather than surveying their field, I hope he will one day give us what he is so well equipped to write: a sustained discussion of machine intelligence and its social context. □

Margaret A. Boden is Professor of Philosophy and Psychology at the University of Sussex. Her books include Artificial Intelligence and Natural Man *(Basic Books, 1977).*

Where are they now?

James S. Trefil

Contact with the Stars: The Search for Extraterrestrial Life. By Reinhard Breuer. Pp.292. ISBN 0-7167-1355-1. (W.H. Freeman: 1982.) $28.50, £11.95.

IT IS HARD to criticize a man who agrees with you. In *Contact with the Stars*, newly translated from the 1978 German publication, Reinhard Breuer presents a view of extraterrestrial life which is becoming something of a new orthodoxy in science: the view that the development of life and of technology is not nearly as straightforward as was thought during the salad days of the 1960s, and that humanity, far from being a junior member of a widespread "galactic club", may, in fact, be a unique feature of our galaxy.

Such a view has many sources, one of which is the pioneering work of Michael Hart on the evolution of planetary atmospheres. Hart showed that the various forces that shape atmospheric development — chemistry, vulcanism, stellar evolution and the effects of life itself — comprise a finely balanced system that would require very little in the way of perturbation to upset it. In the case of the Earth, for example, the atmosphere has been undergoing changes in composition over its four-billion-year history, a period when the brightness of the Sun was increasing by 25 per cent. Life is possible on the Earth only because these two changes took place in exactly the right sequence to cancel each other out. One misstep and the Earth would have experienced a runaway greenhouse effect like Venus or become a frozen ball like Mars or Titan. As I write this, watching my daughters playing in the surf at Cape Hatteras, it is hard to imagine that everything I see — the clouds, the people, the ocean itself — is the result of a happy set of chance occurrences that are unlikely to have been repeated elsewhere. Yet that is precisely what the past 20 years of research on the origins of life tells us.

Another strong argument against the existence of copious numbers of extraterrestrial civilizations is attributed (perhaps apocryphally) to Enrico Fermi. When first presented with the statement that the number of such civilizations in the galaxy could well surpass a million, he is supposed to have remarked "Where is everybody?". The point is that it does not take much imagination to envisage the emergence of humanity as a star-faring race. The idea that we have the technical ability to colonize the entire galaxy is not difficult to accept in the age of space exploration, and calculations tell us that a wave of colonization centred on the Earth would sweep through the galaxy in some 30 million years — a mere blink of the eye in the lifetime of the cosmos. If there really are a million other races out there, most of them will be older and more advanced than we are, so one can argue that the fact that they're not *here* is strong prima facie evidence that they're not *there*, either.

These two types of evidence — one from the study of the origin of life, the other from consideration of the human future in space — have convinced many members of the scientific community that the optimistic estimates of the abundance of extraterrestrial life bandied about in the 1960s and 1970s were simply wrong. As so often happens when new evidence arrives on the scene, old orthodoxies must give way to new. The "galactic club", conducive as it may be to good science fiction, is no exception to the rule.

Contact With the Stars must be understood, then, in the context of the general scientific counter-revolution on the subject of extraterrestrial civilizations. The new ideas have yet to percolate through to the general public, of course, but they are already well known to the experts. The book touches on all of the main topics related to the development of life — stellar evolution, genetic coding, space colonies, UFOs and so on. Some of the topics are treated very well. For example, I got a great deal out of Breuer's discussion of the debate on whether or not we have reached the end of the line in biological evolution. Unlike some who claim that a vastly superior human race will result from genetic engineering, Breuer argues that the genetic code is already carrying just about all the information it can, given the unavoidable copying errors which must occur in replication. This general attitude of informed scepticism is the greatest strength of the book.

On the debit side, the book reads more like a series of unconnected lectures than a single, cohesive document. The author often ventures into asides that range far from the topic under consideration. For example, after having repeatedly asserted his belief that we are alone in the galaxy, he launches into a lengthy and detailed discussion of strategies for communicating with the very extraterrestrials who aren't supposed to be listening. One can only wonder as to why so much effort has been spent devising complex pictographic codes if attempts to communicate are necessarily doomed to failure. ☐

James S. Trefil is a Professor of Physics at the University of Virginia, Charlottesville, and co-author with Robert T. Rood of Are We Alone? *(Charles Scribner's Sons, 1981).*

Books for Christmas

THE next review supplement to be published in *Nature* is Christmas Books, which will appear in the issue of December 9th.

As well as articles on recent science books for children, and on bird books and space science/astronomy books, the supplement will include reviews of *Aha! Gotcha, Darwin for Beginners, More Random Walks in Science, The Cult of the Expert* and *A Geological Miscellany.*

Time as time out of sight and mind

P.W. Atkins

On Time: An Investigation into Scientific Knowledge and Human Experience. By Michael Shallis. Pp.208. ISBN 0-09-148950-4. (Burnett Books/Hutchinson: 1982.) £8.95.

THERE are three types of scientist. One type believes that the whole of human experience is open to scientific investigation and rationalization. Another type believes that there are aspects of the world that lie outside the scope of science. The third type doesn't care one way or the other, and simply gets on with the job. The author of *On Time* is a member of Class 2; I am a member of Class 1, and therein lies a profound disagreement.

Dr Shallis has addressed himself to one of the most elusive aspects of the world: the nature of time. He takes us through a sequence of attitudes to time, ranging from its measurement, through its role in physics (encompassing relativity, entropy and causality), and ends with a series of chapters dealing with what most hard-nosed scientists regard as outside their domain, and lying beyond credulity. All this he treats with an engaging earnestness that fully captures his intention of approaching his subject with "the scientific attitude . . . of a wide-eyed child".

I suspect that you will like this book if you believe that there are aspects of the world outside science; for all *aficionadós* of the paranormal warm to support from within the ranks of practising scientists. I suspect, also, that if you do not believe that science is incapable of dealing with these purported phenomena, such as by dismissing them as hoaxes or by explaining them in terms of established physics, then you will find this book a distressing mish-mash of credulity and passion. What you will not be able to complain about is, as the author so disingenuously reminds us, the impossibility of not going the whole hog on the paranormal once you have embarked on its foothills.

I was particularly struck by one phrase that occurs early on, where Dr Shallis remarks that, by extending conventional "instructional" science to encompass the unreproducible, occasional events that he thinks sometimes obtrude into the world too spasmodically to be captured by scientific method, he provides a description of reality which "will inevitably be richer than the former one". It seems to me that exactly the same remarks are made in favour of the use of hallucinogenic drugs; and with as much force. The heightened richness Dr Shallis asks us to accept (while acknowledging that it may inspire scorn) includes just about everything that may be regarded as symptomatic of crackpots: astrology, precognition, angels and gods (with, I think, fairies and bent teaspoons thrown in for good measure). Some idea of the style of argument is captured by the manner in which the book speaks of α-emission. Apparently, this process is "absolutely causeless" (p.119) except for the possibility that it may be "influenced by mind" (p.125) — particularly, it seems, the minds of young chicks.

I hope that enough has been said to represent without distortion the thrust of Dr Shallis's argument: be totally credulous, swallow the whole of the paranormal, give weight to the hallucinations of the wishful thinking and the downright potty, and disregard the successful continuing progress of modern science. If you accept that argument then you will indeed accompany the author as far as his conclusion, that time is intrinsically paradoxical and must for ever remain elusive. The rejection of that argument, which in the face of the evidence presented here doesn't seem to present much difficulty, keeps open the path to comprehension. ☐

P. W. Atkins is a Fellow of Lincoln College and Lecturer in Physical Chemistry at the University of Oxford. He is author of The Creation *(W.H. Freeman, 1981).*

Breaking the laws

Michael Berry

The Accidental Universe. By P.C.W. Davies. Pp.139. Hbk ISBN 0-521-24212-6; pbk ISBN 0-521-28692-1. (Cambridge University Press: 1982.) Hbk £10, $19.95; pbk £4.95, $9.95.

WHAT would the world be like if the force of gravity were a little stronger? If the proton were a little lighter? It is the purpose of Paul Davies's latest book to study questions such as these and, more generally, to explore the sensitivity of various features of the Universe to the values of the fundamental constants of nature. As groundwork the book begins with an account of the laws of physics and the scales on which they operate, from the shortest "Planck" lengths and times, through those characteristic of nuclear physics, up to cosmological ones.

The main body of the work is devoted to showing how these levels of structure are exquisitely interlocked, so that a change in one level can have astonishing repercussions elsewhere. For example, if the strength of the weak nuclear force (compared to gravity) were slightly different, this would greatly affect energy transport in stars, and probably prevent supernova explosions spewing forth the heavy elements which condense into planets and ultimately ourselves. If the strong nuclear force were a few per cent stronger or weaker there would probably be no stars at all. And if the primordial energy density had been minutely different, the Universe would either have re-contracted implosively long ago (and long before stars and planets could have formed) or exploded so violently as to inhibit condensation into galaxies. Related to this sensitivity are the celebrated "coincidences" involving large numbers; for example, the ratios of the age of the Universe to the time for light to cross a nucleus, and of the strength of gravity to the strength of electromagnetism, are both about 10^{40}.

By examining an impressive variety of such cases (basing his arguments largely on a review article by B.J. Carr and M.J. Rees — *Nature* **278**, 605; 1979), Davies concludes that the Universe is so "fine-tuned" to its present condition by the values of fundamental constants that even a very small alteration would produce a world vastly different from ours and almost certainly unable to support life physically constituted as we know it. This leads him into a discussion of the anthropic principle, which in its weak form asserts: "What we can expect to observe must be restricted by the conditions necessary for our presence as observers", and in its strong form asserts: "The Universe must be such as to admit the creation of observers within it at some stage".

This sudden emergence into cosmology of the idea that the physical structure of the Universe is very special and related to its cognizability is rather strange. It seems to be connected in a curious way with the current preoccupation in applied mathematics with the concepts of genericity and structural stability as especially emphasized by René Thom. Much effort is being expended to determine the nature of the generic (that is, typical, or non-special) solutions of the equations representing physical laws, in the belief that these, being stable against perturbation, can represent persistent structures (condensed matter or life, for example). But the equations themselves are highly non-generic; Maxwell's laws of electromagnetism, for example, are a very special set of linear relations which strongly constrain two vector fields, and any modification would lead to wrong "laws". In this picture of generic solutions of non-generic equations it is difficult to see how the fundamental constants fit in: are they "random initial conditions" or are their ratios determined by laws we do not yet know?

The consequences of physical laws have been explored much more extensively employing the actual ratios of fundamental constants than with hypothetical modified values. It might be that evolution towards structural complexity is virtually irresistible, would survive such modification and still lead to intelligence (albeit with a physical basis quite different from ours — Olaf Stapledon's novel *Star Maker* comes to mind, with intelligence developing via organized fluid motion in the wisps of galaxies and, later, inside stars). If this is correct — that is, if in the space of fundamental constants it could be shown to be generic for universes to have intelligence as an "attractor" of physical laws — the strong anthropic principle would follow as a marvellous consequence, and this aspect of the "coincidences" would lose its mystery.

Paul Davies does not devote much space to such speculations but quite properly concentrates on explaining the physics underlying them. He does this with the lucidity and authority we have come to expect from him. The book should be accessible to anyone with an undergraduate acquaintance with physics, and I warmly recommend it as an excellent introduction to this new and important idea. ☐

Michael Berry is a Professor in the H.H. Wills Physics Laboratory, University of Bristol.

Common ground in a changing climate

Hubert Lamb

The Earth's Climate: Past and Future. By M.I. Budyko. Pp.307. ISBN 0-12-139460-3. (Academic: 1982.) \$39.50, £26.20.

THIS important book by the Soviet Union's outstanding climatologist, Professor M.I. Budyko, is a landmark in the development of climatology as a branch of physical science. At the same time, because climate itself touches more or less every aspect of life on Earth, and because of the way the author draws on the varied works of his long and active career, the book ends by being a statement of Budyko's life philosophy and perhaps of the broadest view of the world as seen from the Soviet Union. As such, it is not only an important work (and one written with notable lucidity) but of wide interest.

Since about 1960 Budyko has been actively involved in concern over man's impact on the environment and with the possibility that the overall effect of the accelerating increase of carbon dioxide in the atmosphere might so disturb the balance of in- and out-going radiation as to lead to an unacceptable warming of the Earth. He was also among the first to discuss the feasibility of counter-measures, such as introducing an aerosol veil into the stratosphere to reduce the warming.

The Earth's climate is some 40°C warmer than would be expected at this distance from the Sun thanks to the extent to which out-radiation is intercepted by the carbon dioxide, water vapour and so on in the atmosphere. Among the most interesting features of Budyko's book is the way in which he traces the changes of climate through the geological past in terms of the carbon dioxide and water supplied by volcanic activity. Decline set in as the volcanism eased off and as vegetation appeared and increased, removing carbon dioxide and converting it to oxygen. This decline was accompanied by a lowering of temperature and the appearance of polar ice. Other shorter-term temperature drops have been associated with periods of specially active volcanism and frequent loading of the atmosphere with veils of dust and aerosol.

However, not all geologists agree that the record of carbon dioxide in the atmosphere fits so well with the known history of prevailing temperatures. Nor can the temperature history of the past century or more be explained by carbon dioxide alone. I am concerned that not only Budyko's book but prevailing opinion among leading meteorologists and climatologists may seriously over-emphasize the importance of carbon dioxide *vis-à-vis* other causes of climatic change. In this book it turns out that in 101 pages carbon dioxide is considered as a major cause of climatic changes, compared with 21 pages for volcanism, and 25 pages for the capacity of polar ice variations and other albedo variations to amplify the overall effect on world temperature. Possible variations of the solar constant get two pages, variations of cloudiness five pages, and continental drift/polar wandering and the Earth's orbital variations about four pages each. The last named variables are surely under-stressed.

The frequent forecasts nowadays, in this book and elsewhere, of a coming drastic change of climate due to man's production of carbon dioxide (and other effluents) are in general a good deal too confident. Despite its basis in the laws of physics, so well expounded in this book, climate is the product of a complex of influences that defies precise modelling. There is no clearly definable limit to the range or the rate of natural climatic variability, particularly the coolings associated with waves of volcanism and (perhaps) other causes. Once within the past 300 years, between 1690 and 1750, there was an oscillation which changed the ten-year average temperatures in England by nearly 2°C, probably the local expression of a global phenomenon. Carbon dioxide changes can be presumed to have played no part in this. The prevailing confidence in the carbon dioxide forecasts is based on their grounding in straightforward physics, but our experience of the difficulties of short-range meteorological forecasts is not entirely irrelevant to this problem.

That said, the possibility of a drastic

climatic warming due to the carbon dioxide has nonetheless to be taken seriously and Budyko has long been pressing upon scientists and politicians alike the urgency of the matter. On the one side, the increase of carbon dioxide could, if its effect were not counteracted by natural or anthropogenic influences, warm world climate by as much as 3°C within about 50 years — and possibly by 10–15°C in the highest latitudes, with disappearance of the polar ice. This and the corresponding changes in the rainfall pattern would be expected to shift the agricultural crop zones and the desert boundary northwards by a highly inconvenient number of degrees of latitude. This would also initiate the rapid melting of the land-based ice-sheets in Greenland and elsewhere, possibly including the great ice-sheet in Antarctica, with a consequent rise of ocean level that would overwhelm the Netherlands and drown large parts of the world's biggest cities. On the other side, forward calculation of the astronomical (Earth's orbital) variations points to a new ice age beginning within about 5,000 years (not the 10,000 to 15,000 years mentioned in the book on the basis of an outdated calculation).

Against this background Budyko sees the increase in carbon dioxide through burning fossil fuel as a benign effect if only it can be moderated to a slower rate of change to which we may be able to adapt. In his eyes man is restoring the atmosphere to its state before the growth of vegetation and is thereby prolonging the possibility of existence of human life and of a biological environment sufficient for our needs. Otherwise the continuing natural decline which set in before the Pliocene would, he suspects, doom the biosphere to extinction within one million years. There are interesting asides in his final chapter about the uniqueness of the Earth's biosphere and the improbability of life, let alone technologically proficient beings, elsewhere in the Universe. On another page, he develops a vision of the future made possible by our postponement of the run-down of carbon dioxide in the atmosphere: a future with a much denser human population and more varied life-forms and, presumably, accelerated through-put of the food chains. Not everyone, however, will see that as progress to a better world.

But in the end there is a noteworthy agreement between the conclusions reached on both sides of the cultural and philosophical divide about our situation in the world today, particularly as regards the threat that man's activities present to our planet. Budyko quotes a Soviet colleague, mentioning a well-known statement by Karl Marx, that

> culture leaves behind itself a desert if it develops spontaneously and is not directed deliberately . . . a developed society ruins nature and destroys the environment from which it derives all its resources.

In this we are no better than our primitive ancestors, whatever their thoughts about the world, who ran so many species of the animals they hunted to extinction. To avert the danger, mankind "must form [take charge of] nature on the global scale". This demand for a cultural dictatorship corresponds to the recognition in informed Western circles of our responsibility towards our successors on Earth and of the need for controls and restraints based on wise counsels and better knowledge. □

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Philosophy in retreat

Stephen Hawking

Cosmology, Physics, and Philosophy. By Benjamin Gal-Or. Pp.522. ISBN 0-387-90581-2. (Springer-Verlag: 1982.) DM 69.

THE ancients considered the ultimate question of "Life, the Universe, and Everything" to be part of philosophy even though they were not sure that the answer was 42. For instance, Aristotle wrote a book on the structure of the heavens in which he put forward good reasons for believing that the Earth was spherical and even gave a figure for the circumference that was correct to within a factor of two. Other philosophers up to and including Kant also considered the structure of the physical Universe to be within the arena of philosophy.

In more recent times, however, philosophers have largely abandoned cosmological questions to physicists and have shrunk the scope of their enquiries to such an extent that many of them now hold that "the sole remaining task for philosophy is the critique of language". Although physicists have brought about great changes in our ideas about the Universe and in our concepts of space and time, they tend to shy away from questions that they regard as philosophical or metaphysical like "does time have a beginning" or "why is the Universe the way it is".

Benjamin Gal-Or, the author of *Cosmology, Physics, and Philosophy*, bemoans the gulf that has grown up between philosophy and cosmology and attempts to bridge it by informing philosophers about the modern theories of physics and by trying to interest physicists in philosophy. In the first of these endeavours he may have some success. The book contains a reasonable account of the hot big-bang theory. It is however overlaid with a lot of other ideas from the modern literature on theoretical physics which the author has included rather uncritically, even though they may not be relevant to the main contents or they may be ephemeral and not taken very seriously by others in the field. An example of this is a flow diagram in the introduction which is meant to show the relations between the main ideas in the book but which is more like a maze with about 40 or 50 boxes connected by arrows. I feel that non-physicists who want to find out about modern theories of the Universe might do better to read the clear and reasonably short book by Steven Weinberg entitled *The First Three Minutes*.

The book's attempt to inform physicists about philosophy is not very successful, at least as far as this physicist is concerned. I found it rather confused and verging on the mystical. One is frequently enjoined to cast aside prejudices and to embrace "gravitism" and "havayism", which is supposed to be the science of the whole, though it was never clear to me what one was supposed to do with them. I also found it rather irritating to have about a quarter of each page in italics for emphasis. Nevertheless, the task that Gal-Or has attempted is a very important one and this book will have to serve until someone writes a better one. □

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In muddied waters

Michael Ruse

The Shaping of Man: Philosophical Aspects of Sociobiology. By Roger Trigg. Pp.186. Hbk ISBN 0-631-13023-3; pbk ISBN 0-631-13028-4. (Basil Blackwell: 1982.) Hbk £12.50; pbk £5.95. To be published in the USA next year by Schocken Books.

THE study of animal social behaviour from an evolutionary perspective dates from the publication of Darwin's *Origin*. But, for a number of reasons — not the least of which is its sheer difficulty — such study languished for a century; and this despite the valiant efforts of a number of workers, most notably and creditably the so-called "ethologists". However, in the past 10–15 years, this member of the family of evolutionary science has awoken, and now races ahead, both theoretically and empirically. Under the new rubric of "sociobiology", the biological basis of animal social behaviour is a rich hunting ground for the brightest and most ambitious of graduate students.

Yet, not everyone has welcomed sociobiology; at least, not everyone has welcomed sociobiology as applied to our own species, *Homo sapiens*. Expectedly, left-wing thinkers are wary of any attempt to relate human behaviour to genes, and they have been joined in their opposition by others. To my regret, members of my own discipline, philosophy, have added their voices to the chorus of dissent, happily "proving" that not only is human sociobiology pernicious and dangerous, but that it is also conceptually impossible! I speak of "regret", because although it is undoubtedly the case that some pretty daft things have been said by advocates of human sociobiology, it surely should be of some moment to us as philosophers that human beings are modified monkeys, rather than the miraculous recent creations of a Being determined to produce offspring in His own image.

The pendulum of opinion now appears to be swinging back, however, and Roger Trigg's new book is happy confirmation of this fact. He is far from an uncritical devotee of human sociobiology, but equally he is convinced that as philosophers — as inquirers into the ultimate nature of man — "we reject the findings of human biology at our peril" (p. xviii).

What is man? Trigg runs through a number of answers and this large survey is one of the irritating aspects of the book; it takes an interminable time before the author turns to his sub-title and to biology. For some one hundred pages we get a Cook's tour, as Trigg takes us rapidly through a selection of the many non-biological answers that have been given to his question. In fairness, the tour is not without value. I am reminded of Woody Allen's comment about how philosophy frustrated him, because he could never tell the difference between heuristics and hermaneutics. He should read Trigg's book, for he will find the solution to that problem, as well as to many others.

Specifically, Trigg introduces us to those such as B.F. Skinner and David Hume who would treat man as object. Some familiar, but nonetheless pertinent objections are raised, for instance the inability of those who regard man as an object to regard themselves as objects! Then Trigg looks at those who take the opposite tack, regarding man as subject. Included here are such thinkers as J-P. Sartre, R.G. Collingwood and H. Gadamer. The difficulties in this approach are noted, including that of ever escaping from the relativism which stems from the overall presuppositions. Next, Trigg looks at sociological approaches to man (his answer is that they are limited), and discusses whether man is a genuine species (yes, but this doesn't tell us as much as we might hope).

And so, finally, we get to the question promised by the book's title: "Is biology the key to the nature of man?". Trigg's answer is guarded. On the one hand, he agrees that biology — specifically human sociobiology — seems to have at least the potential to throw light on human behaviour and its causes; on the other, he warns of the limitations and confusions in the biological approach.

I confess that there were times through this part of his discussion when I felt that Trigg was being a little less than fair to sociobiology (or, at least, to its proponents). Sociobiologists are certainly aware of obvious traps. For instance, if one explains behaviour through (say) kin selection, then although causally one is thinking in terms of reproductive benefits to the individual, it does not follow that one can draw sweeping claims about all humans being "selfish" or some such thing. Talk of "selfish genes", for instance, is metaphorical, and does not commit sociobiologists to the thesis that every human being is a conscious self-interested schemer.

Yet at this point, Trigg accuses sociobiologists of "muddying the waters" for they juxtapose "altruism with an ordinary notion of advantage and self-interest" (p.117). Rather it is Trigg who is muddying waters, or at least who is accusing sociobiologists of being more naive than they really are. Surely he would not suggest that any attempt to give a causal analysis of human behaviour is doomed to failure? Nor even would he suggest that all causes must be related at once to our immediate conscious awareness. Suppose one says that an adult behaves well because of strict childhood discipline. This may or may not be true, but seems to makes sense if the adult does not usually consciously remember the childhood training. If this is so, why is it wrong in principle for sociobiologists to formulate explanations using factors which operate below (or outside) the level of consciousness? And why should they not use metaphors such as "selfish"? Every other scientist uses metaphors (force, work, energy, power, attraction, repulsion and so on).

Finally, Trigg raises the relationship between biology and morality. Again, his conclusion is cautious:

> The insights of sociobiology may give us further understanding of man's nature and give us material on which to base our claims. They cannot take away from us the capability of rational moral choice, based on our conception of the nature of man. The paradox is that any attempt through rational argument to treat man as merely another animal, no different from other animals, is itself self-refuting [p.162].

I'm not quite sure of the strength of this supposed paradox. Is it empirical, in that man simply seems different from other animals? Or is it necessary, in that the very attempt to explain ourselves proves that biology is limited in power? Either way, my own inclination is to agree with Trigg that there is more to morality than biology, in the sense that one certainly cannot deduce morality from biology. Our biological urges are not always "good" urges. Nevertheless, I would go further than Trigg — if indeed we are the product of evolution, then our moral sense presumably has some adaptive value, and hence biology can perhaps throw light on the content of our biology. Biology may not justify in some absolute way our sense of the worth of human being, but it can show why we have such a sense. And perhaps this is all the proof we can ever hope for.

Clearly I don't agree with some of Trigg's conclusions, any more than he would with mine. But that is the nature of philosophy (nor is such disagreement a sign of any inherent weakness in the subject). What counts is that although he is over-long in getting to his main theme, Trigg stimulates one to think on important matters. And that is no small praise. ☐

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Good, bad & biology

Marian Dawkins

The Nature of the Beast: Are Animals Moral? By Stephen R.L. Clark. Pp.127. ISBN 0-19-219130-6. (Oxford University Press: 1982.) £7.95, $14.95.

STEPHEN Clark has made a tactical error. He is a philosopher writing about animal behaviour and attempting to explore the possible analogy between what animals do and human morality. But he unfortunately fails to conceal his contempt for biologists and many of their theories about animals. "The philosopher at a gathering of biologists", he remarks in the Introduction, "is the one in a state of permanent exasperation that these scientists do not understand their own concepts!". He dismisses various biological theories as "merely ridiculous" or "broken-backed and to point this out should be a job for any competent editor, let alone a philosopher".

Writing about a subject which is not your own is always hazardous; but to write in such a way that you raise the hackles of those working in the field before you have even got down to saying what you want to say is hardly the way to persuade biologists that a dose of philosophy would be a good thing. I say this because my own reaction to the book was strongly coloured by resentment at a number of implications, such as that all ethologists are right-wing ideologues who have to be taught a lesson on how to think straight. This resentment probably made me less tolerant of factual errors, more irritated by grammatical awkwardness and far less open to the contribution this book may make than I would have been had exactly the same ideas been put over in a different spirit. For the subject of the book — whether what human beings regard as "moral" has anything to do with what animals have been shaped by natural selection to do — is an important one. There has indeed been a lot of muddled thinking and perhaps facile generalizations from other species to our own. So having expressed my irritation, I will now try to concentrate on content rather than style.

Dr Clark is concerned with what he calls the "morals of Nature". Parent animals care for their children, do not always kill their rivals and apparently respect sexual taboos and figures of authority. There are at least superficial similarities between these examples and conduct which we would call moral if it were done by a human being. But would we be right to describe such behaviour as moral in a non-human species? Are the inhibitions that a wolf feels when it stops short of killing a rival anything like moral ones? And can we learn anything about human morality from studying animals?

To answer these questions, Dr Clark examines various aspects of animal behaviour, including parental behaviour, territory and dominance, drawing on considerable factual detail in the process. He then comes to the conclusions that other animals are not moral in the usual human sense of the word because they do not have the ability to see that they could be wrong. He describes animals as ethical because they "respond to aspects of a situation and to features of their kindred, that a good man would also respect". But he says they are not moral,

> for they do not, as far as we can see, have any occasion to moralise about themselves or to construct intellectual systems to accommodate their immediate responses.

So far Dr Clark's conclusions are easy enough to follow (apart from reservations about some animals which behave in ways which a good man might not respect), but they are neither very surprising nor very different from common sense. It is when he tackles the question of whether human beings should, in any way, adopt the "morals of Nature" that it becomes much more difficult to see what he means. He believes that "the ways of the beasts can set us good examples". Yet he has already argued that the "beasts" are not moral, so it is not clear why he thinks they may set us moral examples. He also believes that some of our moral habits, such as caring for relatives, are "bred into" us. And yet he firmly states that morality does not and cannot come from biology.

One is, in short, left more confused at the end of the book than at the beginning. His scathing criticisms of biologists might have been a great deal easier to stomach if he himself had been able to show that clarity of thought that he feels biologists so obviously lack. ☐

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Separated learning and symbol making

Carolyn A. Ristau

The Unique Animal: The Origin, Nature and Consequences of Human Intelligence. By Don D. Davis. Pp.336. Hbk ISBN 0-907152-02-3; pbk ISBN 0-907152-01-5. (Prytaneum Press: 1981.) Hbk £12.95, $25; pbk £6.95, $12.95.

Don Davis has a provocatively simple hypothesis of the difference between the minds of man and beast. He proposes that man alone is capable of separated learning — of proposing a connection between two or more events separated in time. This entails more than remembering over a long period of time; connecting two seemingly distinct events is essential. As a consequence only human beings can create symbols or discover a relationship between two non-contiguous events, and can hypothesize or propose such connections. Man's hypotheses flower in his magic, religion and science. Man alone has placed a seed in the ground one day, seen a plant grow at that place days later and connected the two events.

But is this the domain only of human beings as Davis claims? Ethologists might note, for example, that certain termites grow fungi and that some ants capture, tend and "raise" aphids for milking, although no one has shown that an individual insect could "discover" the relationships involved. Again, whether man is the only symbol maker seems highly debatable. At least some apes seem to have learned symbols taught by man — as Davis agrees — and some apes' use of novel sign combinations may be indicative of an ability to create a symbol. To conclude that natural animal communication is not symbolic may merely reflect a limitation of our methods of study. For example, the honey bee's dance may be a symbolic achievement when it is used to indicate the location of food.

Equally relevant is the question of whether man is the only creature that in learning can tolerate gaps between environmental events of more than a few seconds. Davis dismisses the obvious counterexamples — taste aversion learning and events that are marked by an especially salient stimulus such as a loud noise — as extremely narrow exceptions. Yet whether animals, like human beings, can "mark" events or locations (an ability that shares characteristics with the process of naming) remains a matter for both philosophical discussion and experimentation. Scent-marking by animals and food-caching with later retrieval may represent rudimentary forms of marking.

To be sure Davis's concept of separated learning *is* powerful, particularly given the way that contiguity as a prerequisite for learning has dominated experimental psychology for decades. No clear examples of such learning by animals come to mind, in contrast with the ease with which Davis is able to apply the concept to pre-historical science, the discovery of fire, of death's inevitability, of smelting, pottery, writing and record-keeping, and the interrelations of magic, religion and science.

Davis's book is of intellectual interest to a broad audience both scientific and popular. There are gaps in his references, however, particularly of pertinent recent research, leading to some incorrect generalizations; and some of his interpretations of research are questionable. But his ideas are important and provocative. They clearly merit attention. ☐

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The pitfalls of bookmaking *(continued from p.112)*

▶ space as an entry in a telephone book. The main thrust (as publishers like to say) is by direct mail which ideally means a leaflet, brochure or booklet landing on the mat of potential customers quickly and reliably. However postal and handling expenses have escalated in recent years and even publishers who specialize in this type of marketing have drawn in their horns somewhat. There can be months of delay before the information about a particular book, especially if it appeals to small groups in different disciplines, reaches all possible readers. Campbell is bound to be on the list which is mailed last.

In the first year of publication perhaps as many as 800 copies will have been sold even though no one seems to know about the book. A further 200 sales will appear on the statement for the following year and then probably no more than another 100 over the next three years. Campbell, a reflective character, may wonder whether this is *the* way to reach the 1,100 purchasers who alone seem to be interested. Where is the electronic revolution? Even publishers stop and wonder, as they see the latest list of overstocks to be "wasted" because they are unsaleable, and ponder whether the print runs should in future be set below 1,000.

Meanwhile, over lunch with Parsons an adviser to the firm suggests there is room for a nice, tightly edited volume on the topic of Campbell's original chapter. The field has grown. The ideal editor would be Campbell himself, and he is approached. Does he accept? ☐

Anthony Watkinson, formerly Editorial Director of Academic Press, London, is now at Oxford University Press.

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Geochemistry of a Pliocene–Pleistocene oceanic-arc plutonic complex, Guadalcanal

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The Koloula Igneous Complex, on the island of Guadalcanal, consists of a low-K calc-alkaline sequence of 26 different intrusive phases. The major intrusions are characterized by $K/Rb > 400$, $Rb/Sr < 0.06$, $\delta^{18}O$ *of 5.7 to 7.2 and uniform* $^{87}Sr/^{86}Sr$ *of 0.70372. This article presents the first data describing oxygen and strontium isotopic behaviour within a plutonic suite that formed by crystal fractionation.*

TECTONIC settings of plutonic rocks (constructive versus destructive margins) in the geological past are often difficult to evaluate because of lack of data from recent plutonic complexes of known tectonic affinities. Such complexes are not commonly found because the very young magmatic terrains must be uplifted and eroded rapidly to expose the plutonic equivalents of the more commonly studied volcanic rocks[1,2]. Older plutonic complexes (such as the Sierra Nevada batholith) do not provide verifiable constraints on tectonic settings and as such are difficult to use for comparisons. We present new data on an oceanic-arc, sub-volcanic plutonic complex that is found on Guadalcanal in the Solomon Islands. Data from this complex can be used as a reference point for the evaluation of the tectonic setting and mantle–crust affinities of older plutonic complexes.

Geological setting

The Solomon Islands form a double *en echelon* straight arc between New Guinea and the New Hebrides island arc (Fig. 1). At present, subduction is from the south-west, with the Australian Plate being consumed beneath the Pacific Plate; although there is evidence that before the late Miocene the situation was reversed, with the Pacific Plate being subducted from the north-east beneath the Australian Plate[3]. The Solomon Islands form an oceanic island arc, and Guadalcanal has been near the site of major plate boundaries since at least the Cretaceous. Basement rocks include Cretaceous tholeiitic lavas, and some schistose equivalents, a Palaeocene gabbro, Oligocene–Miocene basaltic andesites (for example, Suta Volcanics) and their plutonic equivalents (Poha Diorite), and Miocene greywackes derived from basic volcanites[4]. The thickness of the basic crust in Guadalcanal is probably between 14 and 20 km (ref. 5).

The Koloula Igneous Complex (Fig. 2) is situated close to the south coast of Guadalcanal, some 50 km from the axis of the South Solomon Trench, which is the topographic expression of a steeply-dipping subduction zone, to which the Pliocene to Recent volcanic centres of Guadalcanal are genetically related[6]. The Koloula Igneous Complex (age: 4.5–1.5 Myr) is considered to have a similar origin, and is exposed solely because of rapid uplift (at least 2 km since the early Pleistocene) and immense erosion caused by seismically-triggered landslides and heavy rainfall, which at present is variable between 7.5 and 15 m yr^{-1}. Despite dense jungle, present exposure is excellent owing to a dense network of deeply-incised streams. Previous studies have dealt with the petrography and porphyry copper mineralization[7], K–Ar geochronology[8], fluid inclusions[9], and mineralogy[10,11] of the Koloula Igneous Complex.

The Koloula Igneous Complex has intruded the Suta Volcanics of late Oligocene–Miocene age, and consists of a low-K calc-alkaline sequence of 26 different intrusive phases that has been divided into two major magmatic episodes[7]. Cycle 1 intrusions ($\geqslant$4.5 Myr) are, in order of intrusion: Chakachaka Gabbro (including an olivine pyroxenite cumulate), diorite, Vasuata Quartz Diorite, granodiorite and aplite dykes. The latter two silicic units are volumetrically insignificant whereas the gabbro and the quartz diorite are the largest two intrusions, and together comprise about 80% by area of the complex that is exposed at the present time. The gabbro is composed of plagioclase, augite, hornblende, hypersthene, magnetite and commonly 1–2% quartz; olivine is almost unknown. The quartz diorite contains plagioclase, hornblende, quartz, magnetite, trace clinopyroxene, and typically, at least traces of a graphic quartz–alkali feldspar intergrowth.

Cycle 2 intrusions (2.4–1.5 Myr) are divided into the Inamumu Zoned Pluton (IZP) (Fig. 2) and several satellite intrusions. The latter, and six generations of andesitic and doleritic dykes, are not part of the present study. The IZP is composed of six concentrically-disposed quartz diorite and tonalite units, aplite dykes, the Chikora Tonalite Porphyry, and trondhjemite dykes (listed in order of intrusion in Table 1). Quartz diorites and tonalites of the IZP contain plagioclase, hornblende, quartz, biotite and magnetite, and except for tonalites 2 and 3, are typically devoid of K-feldspar.

Major- and trace-element data

The variations of major- and trace-element contents of whole rocks versus silica describe smooth and slightly curved trends over the interval 45–76% SiO_2 and are considered to be due to crystal fractionation[12] (Fig. 3). These trends are caused initially by fractionation of amphibole and then by biotite, an observation similar to that noted by Taubeneck[13] for other tonalitic intrusions. P_2O_5 content exhibits a smooth convex-upward shaped trend, typical in magmatic suites formed by crystal fractionation of apatite[14]. The gabbroic rocks are generally strongly leucocratic as they are the residual fraction after the separation of the olivine pyroxenite unit. Their analyses display a much wider compositional scatter than those of the more siliceous rock types and this behaviour is probably related to variable amounts of pyroxene and olivine segregations in some gabbro samples.

With the exception of the late-stage aplite dykes present in both magmatic cycles, there is a compositional overlap of rocks from cycles 1 and 2 between 53 and 64% SiO_2 (based on 110 whole-rock analyses[12]). The major rock-types are characterized by low abundances of K (250–12, 750 p.p.m.), Rb (1–46 p.p.m.), and Ba (207–493 p.p.m.); high Sr (360–700 p.p.m.), moderately high K/Rb and K/Ba ratios and low Rb/Sr ratios. K/Rb and K/Ba ratios are lower for cycle 2 than for cycle 1. Some representative analyses are given in Table 1 and shown in Fig. 4.

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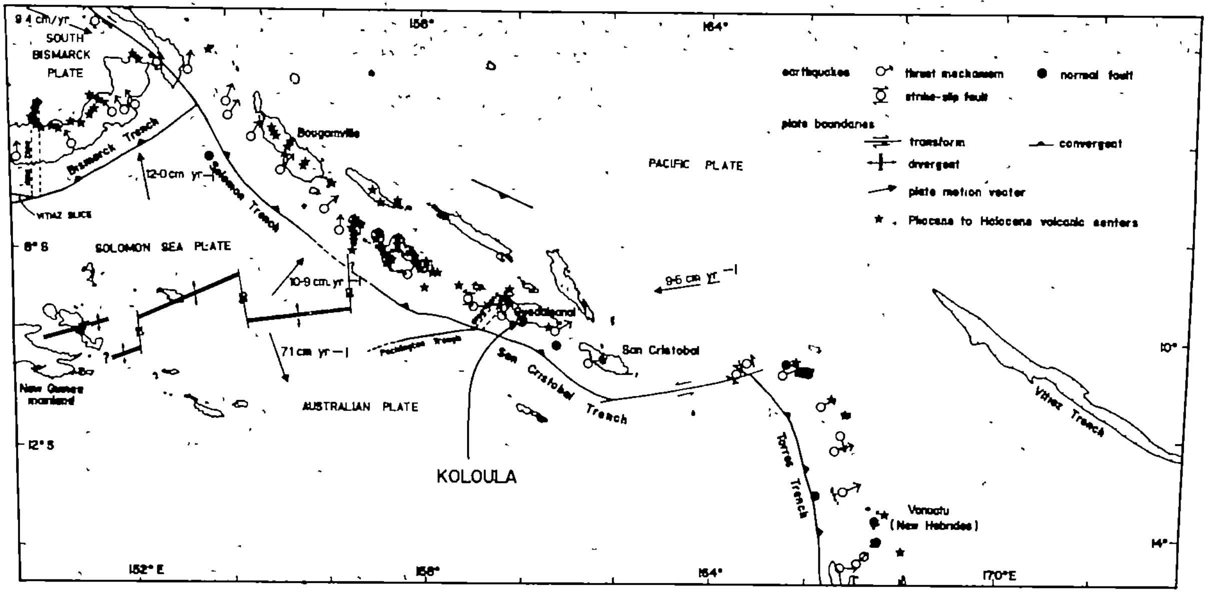

Fig. 1 Location map and tectonic synthesis of the Solomon Islands[7].

Strontium and oxygen isotope data

Rocks and minerals from plutons belonging to both cycles 1 and 2 have been analysed for oxygen and strontium isotopic compositions (Table 1). The alteration associated with fluid circulation peripheral to known prophyry copper mineralization in the Chikora Tonalite Porphyry[6] has clearly affected oxygen and hydrogen isotopic compositions in altered rocks. For example, sample A.276, a sericite + calcite + chlorite + pyrite filled fracture has low $\delta^{18}O = 3.6$ and high $\delta D = -48$ which are commonly measured values in rocks affected by hydrothermal fluids. Isotopic evidence of hydrothermal alteration can also be seen in some samples (A.628, A.838, A.41) with less obvious petrographic evidence of alteration (see ref. 15). Unlike $\delta^{18}O$ values, $^{87}Sr/^{86}Sr$ ratios from altered samples are the same as values of unaltered rocks or are only slightly higher (0.7037–0.7038). If the alteration fluid were seawater, as has been previously inferred[7] and as suggested by $^{87}Sr/^{86}Sr$ ratios of the alteration minerals (laumontite and calcite), then the small shift in $^{87}Sr/^{86}Sr$ ratios is a reflection of low water/rock (W/R) ratios and the high strontium contents of the rocks (500–600 p.p.m.) relative to that of seawater (8 p.p.m.)[16]. Strontium and oxygen isotope compositions may be used together to determine W/R ratios and temperatures of water–rock equilibration[17]. For closed-system exchange, the strontium W/R (by weight) between seawater and rock, is given by:

$$W/R = \frac{^{87}Sr/^{86}Sr^{f}(\text{rock}) - {}^{87}Sr/^{86}Sr^{i}(\text{rock})}{^{87}Sr/^{86}Sr^{i}(H_2O) - {}^{87}Sr/^{86}Sr^{f}(\text{rock})} \times \frac{C^{i}(\text{rock})}{C^{i}(H_2O)}$$

where i = initial, f = final, C^i(rock) = concentration of strontium in rocks and $C^i(H_2O)$ = concentration of strontium in seawater. W/R ratios of 4 are calculated for completely altered rocks using data from calcite and laumontite veins where $^{87}Sr/^{86}Sr^f = 0.7040$ and the parameters $^{87}Sr/^{86}Sr^i(\text{rock}) = 0.7037$, $^{87}Sr/^{86}Sr^i(H_2O) = 0.709$, $C^i(H_2O) = 8$ and C^i (rock) = 550. The strontium contents of altered rocks and minerals (200–400 p.p.m.) are lower than those of unaltered rocks (500–600 p.p.m.) and suggest loss of strontium to the alteration fluid and that the system was not entirely closed. The assumption of closed system behaviour maximizes the calculated W/R ratio. At such low W/R ratios and high concentrations of strontium in the altered rocks, the shifts in $^{87}Sr/^{86}Sr$ composition associated with alteration are at the limits of our detection and without the oxygen-isotope data would probably go undetected. As such, use of alteration-related minerals (laumontite, calcite) provides evidence of strontium isotopic exchange that is well within analytical resolution. Using the W/R ratio obtained

Table 1 Geochemical and isotopic data for selected samples from the Koloula igneous complex

	Sample no.	SiO_2 (%)	Na_2O (%)	K_2O (%)	Rb (p.p.m.)	Sr (p.p.m.)	Ba (p.p.m.)	Rb/Sr	K/Rb	K/Ba	$^{87}Sr/^{86}Sr$	$\delta^{18}O$ (‰ SMOW)
Cycle 1												
Olivine pyroxenite	AA.3	49 54	0.50	0.04	0.7*	164	11	0.004	714	30	0.70374±8	5.6
Chakachaka Gabbro	A.962	46.39	1.80	0 13	2.1*	695*	—	0.003	514		0.70374±12	5.4
Vasuata Quartz Diorite	A.628	56.68	4.16	1.09	15	455	207	0.033	603	44	0.70370±8	5.3
Granodiorite	A.838	63 50	4.09	3.20	46	359	493	0.128	577	54	0.70370±8	4.0
Aplite dyke	A.723	75.93	3.77	4.56	64	210	632	0.305	591	60	0.70373±8	7.2
Cycle 2 Inamumu Zoned Pluton												
Charilava Quartz Diorite	A.471	52.83	2 87	0.71	12.8*	59.6*	—	0.021	460	—	0.70378±8	5.7
Charilava Quartz Diorite	A.421	56.30	3.34	0.99	20	602	219	0.033	411	38	0.70371±6	6.0
Tonalite 1	A 356	60.70	3.47	1.16	24	581	274	0.041	401	35	0.70371±8	6.1
Porphyritic tonalite	A.118	62.71	3.80	1.39	31	626	327	0.050	540	35	0.70376±12	6.2
Tonalite 2	A.84	64.02	3.63	1.25	27	652	351	0.041	384	30	0.70368±8	6.7
Tonalite 3	A.82	65.24	3.77	1.53	37	635	409	0.058	343	31	0.70374±8	6.6
Aplite dyke	A.6	76.67	3.09	4 93	58	203	765	0.286	1,024	53	0.70372±20	6.9
Chikora Tonalite Porphyry	A.41	67.77	4.42	0.87							0 70363±10	6.1
Trondhjemite dyke	CH.8-622.5	75.83	4.58	1.10								7 2
Altered rocks and minerals												
Pervasive sericite–calcite–chlorite–pyrite zone in Charilava Quartz Diorite	A.276	51.14	0.81	3.79	107	191	427	0.560	294	74	0.70376±8	3 6
Laumontite vein	A.479					380*					0.70404±8	
Calcite vein	IHP.493										0.70401±12	

* Concentration determined by isotope dilution. All other abundances determined by X-ray fluorescence analysis. Strontium isotopic ratios have been normalized to E & A standard value of 0 7080; errors are reported as standard error of the mean at 2σ.

Table 2 Comparative geochemical and isotopic data for rocks from oceanic and continental terrains

		SiO_2 (%)	K (p.p.m.)	Rb (p.p.m.)	Sr (p.p.m.)	Rb/Sr	K/Rb	$^{87}Sr/^{86}Sr$	Refs
Oceanic									
Koloula	Cycle 1								
	Gabbro	46.4	1,079	2.1	695	0.003	514	0.70374	
	Quartz diorite	56.7	9,049	15	455	0.033	603	0.70370	
	Cycle 2								
	Tonalite	60.7	9,628	24	581	0.041	401	0.70358	
Tanzawa	Gabbro	43.1	585		280	0.0035	585	0.70345	21
	Tonalite	62.1	4,553	8	251	0.032	569	0.70367	
Viti Levu	Gabbro	48.7	1,410	2	190	0.010	705	0.7037	22
	Tonalite	64.4	5,560	8	349	0.023	695		
Captains Bay	Gabbro	48.4	996	3	640	0.005	333	0.70377	24
	Quartz diorite	59 3	12,450	34	426	0.08	368	0.70317	
Continental									
Chile (Tertiary)	Tonalite	62.5	13,860	45	465	0.10	308		26
USA Cloudy Pass	Quartz diorite	61.4	19,670	65	288	0.23	303	0.7047	29

above and the equation for closed-system oxygen isotope exchange of seawater and rock (by weight)[15]:

$$W/R = \frac{\delta^f(\text{rock}) - \delta^i(\text{rock})}{\delta^i(H_2O) - (\delta^f(\text{rock}) - \Delta)} \times \frac{C^i(\text{rock})}{C^i(H_2O)}$$

we can obtain values for Δ. For the alteration of the Charilava Quartz Diorite with seawater we obtain a value of $\Delta = 3.4$. Using the feldspar-H_2O geothermometer[18] ($\Delta = 2.68 (10^6/T^2) - 3.53$) we calculate a temperature of 350 °C for water–rock equilibration. This agrees well with the fluid inclusion filling temperature of 350 °C obtained on rocks from the same B4 alteration zone[9].

Using petrographic criteria and oxygen isotope compositions to eliminate altered samples, the unaltered rocks from cycle 1 have $\delta^{18}O$ values that range from 5.4 for a gabbro to 7.2 for an aplite dyke whereas $\delta^{18}O$ values from the IZP (cycle 2) range from 5.7 (quartz diorite) to 7.2 (trondjhemite dyke). The observed variations in $\delta^{18}O$ values (5.4–7.2) and SiO_2 contents (46–77%) fall along a well defined line with a slope of 0.06‰/% SiO_2 (Fig. 5). For these samples, $^{87}Sr/^{86}Sr$ ratios range from 0.70368 to 0.70378 ($\bar{x} = 0.70372 \pm 8$ at 2σ) for the entire range of SiO_2 and are identical within experimental errors.

The lack of variation in strontium isotopic ratios and the correlation of $\delta^{18}O$ with degree of differentiation (given by %

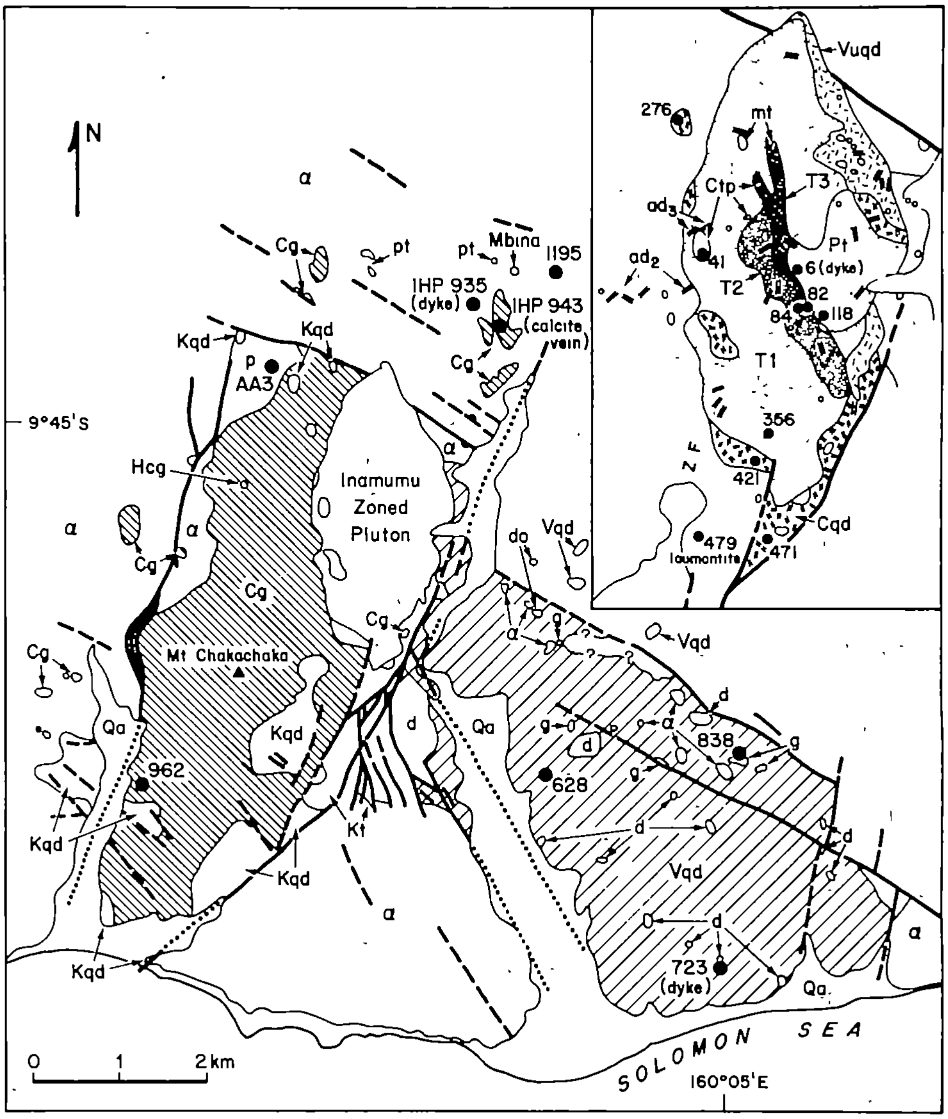

Fig. 2 Geological map of the Koloula Igneous Complex showing sample locations. Faults are shown as both heavy dotted and solid lines. Cycle 1 rock types: Cg, Chakachaka Gabbro; p, olivine pyroxenite; d, diorite; Vqd, Vasuata Quartz Diorite; do, dolerite; g, granodiorite. Cycle 2 satellite plutons: Kqd, Kolochoro Quartz Diorite; Kt, Kolokemau Tonalite; Hcg, High Chikora Granodiorite. a, Suta Volcanics (Oligocene to early Miocene); Qa, Holocene alluvium; Q, Inamumu Zoned Pluton (inset): Vuqd, Vurakara Quartz Diorite; Cqd, Charilava Quartz Diorite; T1, tonalite 1; Pt, porphyritic tonalite; T2, tonalite 2; T3, tonalite 3; mt, melanocratic tonalite; Ctp, Chikora Tonalite Porphyry. Aplite and hornblende andesite dykes are not shown. ZF, zone of foliation.

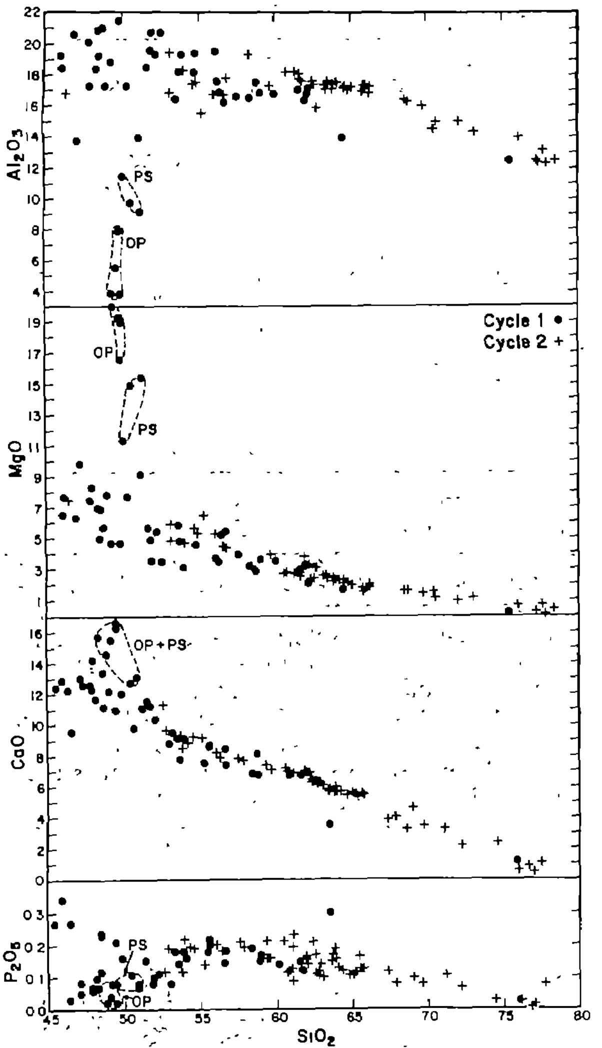

Fig. 3 Harker variation diagrams for selected major elements for samples from the Koloula Igneous Complex. OP, olivine pyroxenite; PS, pyroxenite segregation. Five samples of unusually mafic-rich gabbro provide a trend of high P_2O_5 contents between $SiO_2 = 45\%$ and 50%.

SiO_2) is indicative of temperature-dependent fractionation of oxygen isotopes. The magnitude of oxygen isotope fractionation between cumulus and liquid phases is dependent on the temperature of crystallization and the mineralogy of the cumulate hases in equilibrium with the liquid. As such, the slope of the correlation line on the SiO_2 versus $\delta^{18}O$ diagram for igneous rocks that have formed by simple crystal fractionation, will not be the same for all plutonic and volcanic complexes. However, for all igneous suites that have originated in the mantle, the correlation line should pass through a common field. In a suite of tholeiitic lavas from the Hachijo-jima volcano in the Izu-Mariana arc, Matsuhisa[19] found a $\delta^{18}O$ variation from 5.7 to 6.7‰ concomitant with a variation in SiO_2 of 47–73% (0.04‰/% SiO_2).

Comparisons with other epizonal plutons

Although well documented studies of subvolcanic magma chambers are rare, we have selected five examples that are probably related to arc magmatism (Table 2). Two of the areas are considered oceanic, two are continental and one, transitional from oceanic to continental. The oceanic plutonic complexes from Tanzawa, Japan and Viti Levu, Fiji are similar to the Koloula complex, in rock type (gabbro-tonalite) and geochemical data (Fig. 4). The Tanzawa complex is composed primarily of gabbro-tonalite plutons of Miocene age and has been interpreted as a low-K, island-arc plutonic complex[20,21]. The strontium isotope data from Tanzawa appear to be slightly anomalous in that the tonalites have an average $^{87}Sr/^{86}Sr$ ratio of 0.7036 whereas the gabbros give values of 0.70345. Although data are available from Viti Levu, it is obvious that significant alteration (10–20 modal % of alteration minerals) has caused considerable scatter[22]. Other geochemical characteristics (K, Rb, Sr contents) of the igneous complexes from both Tanzawa and Viti Levu are consistent with their having been derived from oceanic mantle with no crustal component. Measured K/Rb (>400) and Rb/Sr (<0.06) ratios are similar to volcanic rocks from other oceanic island arcs (for example, Tonga[23]). The Captains Bay pluton, Unalaska Island, Alaska[24] is zoned inward from gabbro to monzodiorite. Even though the $^{87}Sr/^{86}Sr$ ratios are similar to Koloula (0.70377), the low K/Rb (333–368) and the high Rb/Sr (0.08) for the quartz diorite are different. Based on conclusions of Perfit *et al.*[24] we have included the data under our oceanic plutonic complexes, but it is likely that Unalaska Island may straddle the continental–oceanic environments[25].

The continental-margin plutonic complexes related to subduction from both Chile[26] and western USA (Cloudy Pass[27] and Tatoosh[28,29]) are mostly tonalitic in composition and show consistently high K (>13,000 p.p.m.) and Rb (>42 p.p.m.) contents, high Rb/Sr (>0.08), and low K/Rb (<333). Lines with a Rb/Sr value of 0.06 and K/Rb ratio of 400 can be drawn in Fig. 4 that effectively separate oceanic and continental terrains. The limited strontium isotopic data (>0.7040) suggests a crustal component in the evolution of these plutons.

Except for Captains Bay, there is a notable lack of stable-isotope data for the examples cited so that we have little indication of the degree of alteration and its effects on other geochemical parameters. The large variation of $\delta^{18}O$ values from Captains Bay[24], of −4.1 to +5.7 is reflective of variations in $^{87}Sr/^{86}Sr$ ratios of 0.70328–0.70377 which can be caused by hydrothermal alteration.

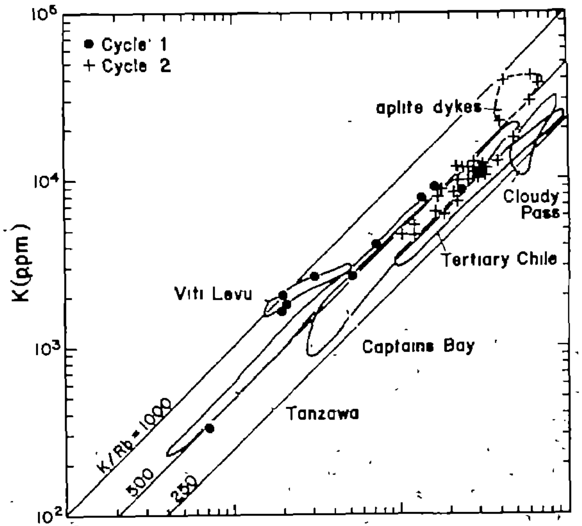

Fig. 4 K–Sr–Rb diagram for the Koloula Igneous Complex. Also shown are fields of gabbro to tonalite rocks from Tanzawa[21], Viti Levu[22], Captains Bay[23], Tertiary tonalites from Chile[26] and Cloudy Pass[29].

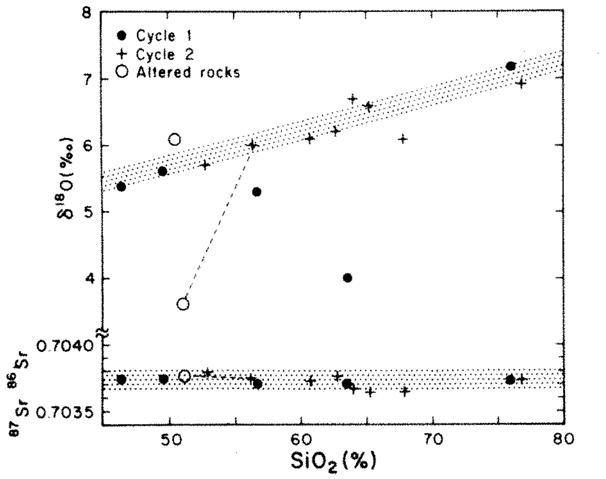

Fig. 5 Variation in whole-rock $\delta^{18}O$ values and initial $^{87}Sr/^{86}Sr$ ratios with % SiO_2 for samples from the Koloula Igneous Complex. Petrographic observations were used to characterize alteration. Tielines join altered and unaltered samples from the same intrusive phase.

Interpretation

The Koloula Igneous Complex is composed of 26 intrusive phases that range from gabbro to aplite. The youthfulness of the complex, its excellent exposure and our knowledge of its tectonic origins make the Koloula study unique in the geological literature. The geochemical variations seen within the two cycles of igneous intrusion are considered to be due to crystal fractionation. Within each cycle K/Rb and K/Ba ratios are similar and suggest that the members of each cycle are comagmatic. The difference in K/Rb and K/Ba ratios between cycles, with no difference in $^{87}Sr/^{86}Sr$ ratios and $\delta^{18}O$ values suggests a similar source for the magmas but different degrees of partial melting.

The large range in composition and relatively low and constant $^{87}Sr/^{86}Sr$ ratios observed in these rocks also makes it possible to model variations in oxygen isotope composition as related to crystal fractionation. Although such variations have been noted in volcanic rocks from Hachijo-Jima in the Izu-Mariana group[21], our data are the first to demonstrate these isotopic systematics in plutonic rocks resulting purely from crystal fractionation of a mantle-derived magma in a closed system. From our data which display relatively low $\delta^{18}O$ at high SiO_2 contents it becomes increasingly evident that examples of the more potassic suite, gabbro → granodiorite → granite are not a result of closed-system differentiation in either small (for example Ploumanac'h[30]) or large plutonic bodies (for example, Peninsular Ranges[31]). The evolution of these granites involves magma contamination by continental crust or partial melting of mixed mantle/continental source regions. Volcanic rocks from the Banda Arc also exhibit a correlation of $^{86}Sr/^{86}Sr$ with $\delta^{18}O$ that has been interpreted as being the result of contamination by crustal material or by subducted sediments[32].

From the comparisons with the limited data available from other young island-arc plutonic complexes, it appears that in unaltered complexes distinctions that are based on trace-element chemistries and isotopic ratios may be made between oceanic and continental environments.

Before this can be applied rigorously to plutonic complexes of uncertain tectonic environment, we need many more detailed studies of young complexes in known tectonic terrains. When this data base is established it may be then applied to older orogens where plutonic rocks are commonly the only remnants of volcanic activity.

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Mechanism of activation of a human oncogene

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The oncogene of the human EJ bladder carcinoma cell lines arose via alteration of a cellular proto-oncogene. Experiments are presented that localize the genetic lesion that led to activation of the oncogene. The lesion has no affect on levels of expression of the oncogene. Instead, it affects the structure of the oncogene-encoded protein.

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A LARGE body of work on chemical carcinogenesis has demonstrated that the carcinogenic potency of a compound is correlated with its mutagenic powers[1-4]; this suggests that DNA is the ultimate target of carcinogenic activation. Accordingly, we and others initiated a series of experiments designed to identify and study DNA segments in tumour cells whose alteration is critically important for oncogenic conversion[5-9].

An initial approach to this problem involved the transfer of tumour cell DNA into non-transformed NIH 3T3 mouse fibroblasts. Such experiments indicated that the phenotype of cellular transformation could be passed from cell to cell in this manner. Thus, the tumour DNA was able to induce foci of transformed cells in a recipient NIH 3T3 monolayer culture while DNA of normal, untransformed donor cells failed to do so[5-9]. These results demonstrated the existence of oncogenic factors which were present in tumour cell DNA and apparently absent from the DNA of normal cells.

That specific donor DNA sequences were involved in these phenomena was first indicated by studies examining the sensitivity or resistance of the biological activity of the DNA to treatment by various site-specific endonucleases[10,11], and was later directly demonstrated by molecular isolation of discrete transforming genes from human bladder carcinoma cell lines[12-14]. Use of clones of the bladder oncogene as sequence probes showed that the oncogene derived from a sequence of similar structure present in the normal human genome[12-14]. It appeared, therefore, that the bladder oncogene had arisen by the mutation of a normal cellular gene during the process of carcinogenesis. The present report addresses the nature of the alterations that activated the oncogene of the EJ human bladder carcinoma cell line.

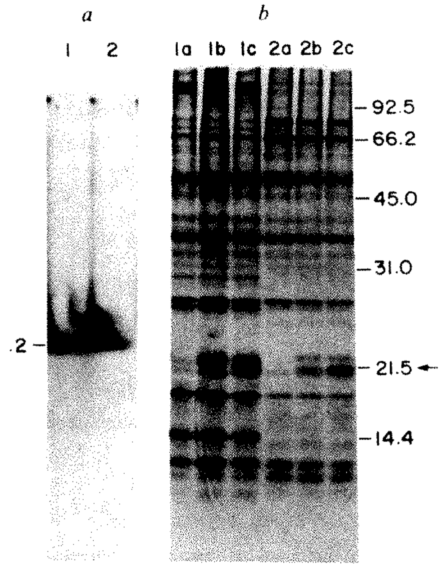

Fig. 1 Comparison of expression of the c-Ha-*ras* gene in normal human bladder epithelial cells with that in the EJ transformed bladder cell line. The normal bladder epithelial cells, strain HB1-5, were secondary and tertiary passage cultures derived from explants of a 5-month fetal human bladder. The cells were cultured as described[23]. *a*, The relative levels of c-Ha-*ras* specific RNA in the two cell types: lane 1, RNA from EJ cells; lane 2, RNA from Hbl-5 cells. Total polyadenylated RNA was prepared by the technique of Varmus *et al.*[40]; 4 μg of RNA was then fractionated by electrophoresis through formaldehyde-containing 2% agarose gels and transferred to nitrocellulose (B. Seed & D. Goldberg, manuscript in preparation). A *ras*-specific probe was prepared by cutting pEJ with *Bam*HI, fractionating the resultant fragments through a 1% agarose gel and extracting the 6.6-kb insert with NaI and glass beads. The nick-translated fragment (6.6×10^7 c.p.m. μg^{-1}) was annealed to the immobilized RNA[41,42]. Bands hybridizing to the probe were visualized by autoradiography. Molecular weights were determined by comparison with markers obtained from *in vitro* run-off transcription of the adenovirus late promoter[43]. *b*, A comparison of p21 proteins immunoprecipitated from cell lysates of EJ cell clones (lanes 1, a–c) and HB1-5 cells (lanes 2, a–c). Cultures were labelled with ^{35}S-methionine for 12 h. Lysates were then prepared and immunoprecipitated with non-immune serum (lanes 1a and 2a), a monoclonal antiserum (Y13-238) which precipitates the p21 encoded by Ha-MuSV but not the p21 encoded by Ki-MuSV (lanes 1b and 2b) or a monoclonal antiserum (Y13-259) which detects both the Ha-MuSV and the Ki-MuSV p21s (lanes 1c and 2c)[25,32]. 20×10^6 c.p.m. of lysate per sample were resolved by electrophoresis through a 12.5% SDS-polyacrylamide gel.

Comparison of this EJ bladder oncogene with the related cellular sequence (the proto-oncogene) was aided greatly by the subsequent discovery that this oncogene was homologous to the transforming gene of the rat-derived Harvey murine sarcoma virus[15-17]. This rat sarcoma virus gene, termed v-Ha-*ras*, had been acquired from the rat genome during the process of formation of the chimaeric viral genome[18,19]. Both the rat and human cellular homologues of v-Ha-*ras* have been isolated in the course of studies of this gene[20,21]. The human cellular homologue of the v-Ha-*ras* was found to correspond precisely to the normal antecedent of the EJ bladder oncogene[16].

From examination of the data in these cited reports, it was possible to make preliminary comparisons between the EJ oncogene[13] and its normal cellular counterpart (termed c-Ha-*ras*)[22]. For example, a molecular clone of the normal cellular gene did not induce foci when applied to NIH 3T3 monolayers, while a clone of the bladder oncogene exhibited a biological activity of $\sim5\times10^4$ focus-forming units per μg of transfected DNA[13,21]. This stark difference in function did not correlate with any apparent structural differences between the two clones. Rough restriction endonuclease site mapping of the EJ oncogene clone and the uncloned related human proto-oncogene indicated that the two were basically indistinguishable over the 6.6 kilobase (kb) of sequence which contained the transforming activity of the EJ oncogene[13]. Finer mapping was later made possible by the direct comparison of molecular clones of the two genes. We again found no differences using a series of different endonucleases, with the exception of a single difference 3′ (downstream) of the coding regions of the genes (data not shown). This latter difference was interpreted to represent a functionally silent polymorphism of the gene present in the human gene pool. Such polymorphisms of this gene have been documented independently by others[12].

These results presented an apparent paradox, since a drastic functional difference was exhibited by two structurally similar genes. We assumed that a minor alteration was responsible for the functional difference, and that this alteration could affect function in one of two ways: the alteration could involve a change in sequences regulating the expression of the gene; alternatively, the transformed phenotype could be due to changes in the protein-encoding portion of the gene. The first hypothesis would argue for up-regulation of transcription or translation of the gene, yielding high levels of an otherwise normal protein product, while the second model would suggest synthesis of an altered protein. Both types of alteration could also act in concert to create the observed difference in function.

Analysis of oncogene activity in normal and transformed bladder cells

If the significant difference between the oncogene and proto-oncogene were one of regulation, one would expect to see differences in levels of RNAs and proteins specified by these genes. To address this question, we first examined the EJ bladder carcinoma cells and their counterpart normal cells. Normal bladder epithelial cells were cultured from a specimen of normal human bladder as described[23], and were found to synthesize keratins, require a fibroblast feeder layer support, and to possess a limited lifespan in culture, as would be expected for normal epithelial cells (T. O'Connell and J. G. Rheinwald, personal communication). The cultures were free of human stromal fibroblasts, and were purified free of feeder cells before nucleic acid extraction. These cultured cells therefore provided a source of genetic material and RNA transcripts that were the normal counterparts of the bladder carcinoma genes and transcripts.

Total cellular RNA was prepared from both normal and transformed bladder cells. Transcripts were analysed by running the RNA on a formaldehyde gel, transferring it to a nitrocel-

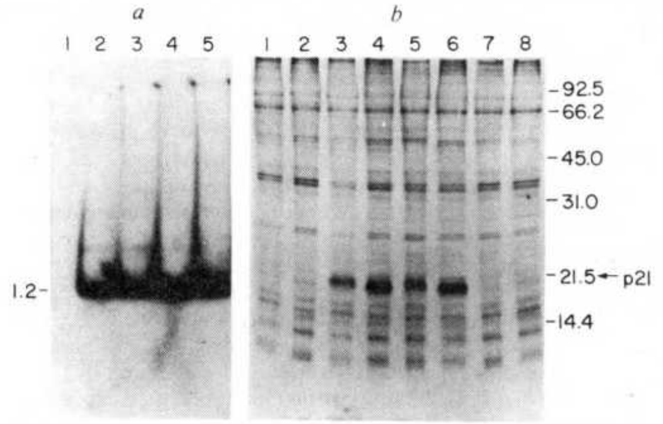

Fig. 2 Analysis of cells transfected with molecular clones of the EJ transforming gene (pEJ) or its normal cellular homologue (pEC). Transfections were carried out as previously described[44,45] using 75 μg NIH 3T3 carrier DNA, 500 ng pEJ or pEC DNA, and 50 ng pEcogpt DNA per 2×10^6 cells. Colonies resistant to mycophenolic acid were selected[26]. *a*, Analysis of total polyadenylated RNA from the four transfected cell lines. RNAs were isolated and analysed as described in Fig. 1. Lane 1, RNA from NIH 3T3 cells; lane 2, EJ/Gpt-2 cells; lane 3, EJ/Gpt-3 cells; lane 4, EC/Gpt-1 cells; lane 5, EC/Gpt-5 cells. *b*, A comparison of p21 proteins immunoprecipitated from cell lysates of the four lines transfected with pEJ or pEC. Cell lysates were prepared, immunoprecipitated and analysed as described in Fig. 1 legend. Immunoprecipitations were performed with non-immune serum (lanes 1, 2, 7, 8) or the monoclonal antiserum (Y13-238) which precipitates the Ha-MuSV p21 (lanes 3–6). Cell lysates were prepared from EJ/Gpt-2 (lanes 1, 3); EC/Gpt-1 (lanes 2, 4); EJ/Gpt-3 (lanes 5, 7) and EC/Gpt-5 (lanes 6, 8).

lulose filter, and probing the filter with nick-translated EJ oncogene clone. Figure 1*a* shows that similar levels of RNA were detected in the two cultures. The transcripts were of a size (1.2 kb) previously associated with the c-*ras* genes of other cell types[24].

The only known products of the *ras* genes are proteins of molecular weight ~21,000, referred to as p21. Monoclonal antisera against the v-Ha-*ras* p21 protein[25] were used to precipitate metabolically labelled protein lysates from both EJ and normal bladder cells. Control experiments ensured that the amounts of antibody used in this and subsequent experiments were in excess of that required to immunoprecipitate the antigen present. The data shown in Fig. 1*b* indicate that at least two bands of radiolabelled protein were specifically precipitated by the anti-p21 sera from normal bladder cells. Detailed examination of the protein pattern of the bladder carcinoma seen in this and other gels reveals a more complex array of bands. On close inspection we believe that four bands in total can be discerned. Data to be presented below clarify the origin of the novel p21 bands seen in the EJ tumour cells. After comparing the intensities of the p21 bands with those of non-specifically precipitated background bands, it became apparent that the total amount of p21 proteins of the normal and the tumour cells differed less than threefold.

It thus seems that increased levels of transcription are not responsible for the novel activity exhibited by the EJ oncogene. This conclusion rests in part on the fact that in the hybridization conditions used here, the oncogene probe reacts exclusively with transcripts of the human c-Ha-*ras*-1 gene. Interpretation of the protein data is less clear. It is apparent that both cells have comparable levels of proteins that are reactive with the Harvey-specific serum, and that these proteins can be collectively termed 'p21'. The nature of these proteins can only be addressed by other studies, presented in part below.

Analysis of gene activity in cells transfected with cloned DNAs

It remained possible that the bladder epithelial cells were not, as we believed, representatives of the normal precursors of the bladder carcinoma cells. Such a possibility might cloud interpretation, as a *ras* gene could be expressed at a high level in one cell type without inducing transformation, and only achieve this phenotype when inappropriately expressed in a second cell type. We therefore wished to measure the levels of transcription and translation of the two genes in the same cellular background.

We therefore introduced molecular clones of both genes into NIH 3T3 cells. Colonies acquiring the EJ oncogene could be readily identified by their transformed morphology. However, cells acquiring clones of the normal allele were not identifiable by any obvious change in behaviour. Because of this, we co-transfected a clone of the dominant selectable *Ecogpt* gene[26] into NIH 3T3 cells together with a 10-fold excess of either the cloned EJ oncogene (pEJ) or the cloned proto-oncogene (pEC). In each case, colonies were selected for resistance to mycophenolic acid imparted by acquired *Ecogpt* genes[26]. This strategy was used since the introduction of a non-selected DNA segment can be ensured by co-transfection with a selectable gene[27]. In the present case, 75% of the mycophenolic acid-resistant colonies deriving from co-transfection of *Ecogpt* and pEJ were seen to be morphologically transformed; as expected, none of the colonies emerging after co-transfection with pEC was transformed.

The cellular DNA of both classes of colonies was analysed for the presence of pEC or pEJ sequences. DNA was cleaved with the restriction enzyme *Bam*HI, which would be expected to liberate a 6.6-kb fragment from each intact copy of the cloned oncogene or proto-oncogene. The normal mouse homologue of the *ras* gene hybridizes only weakly to the pEJ probe[16], and so its presence does not obscure the analysis. To ensure that transfected pBR322 sequences would not interfere with interpretation of the data, *ras*-specific sequences were prepared from pEJ and used as probe; 75% of non-transformed colonies transfected with the proto-oncogene and all of the transformed, oncogene-transfected colonies showed the presence of pEJ-homologous sequences migrating at 6.6 kb (data not shown).

We selected for further analysis two cell lines containing intact copies of the oncogene and two lines containing an approximately equal number of intact copies of the proto-oncogene. Photographs of these cell lines are shown in Fig. 3; 4×10^6 cells of each of these lines were injected into 6-week-old NSF mice. Each of the pEJ transfected lines elicited tumours in 5/5 mice, while none of the mice injected in parallel with pEC transfectants developed tumours.

Total cellular RNA was prepared from all four transfected cell lines. The RNA preparations were then run on a formaldehyde gel, transferred to nitrocellulose filters, and probed with the *ras*-specific DNA. As shown in Fig. 2*a*, the level of *ras*-specific RNA in cells containing the oncogene was comparable with those carrying the transfected proto-oncogene. We also examined the levels of p21 in these cell lines. Figure 2*b* shows that monoclonal antiserum against p21 precipitates similar amounts of p21 protein in pEC and pEJ transfected cells.

These data do not address the question of whether the two cloned genes are transcribed at the same rates in these cells, as it remains formally possible that few of the acquired copies of the pEJ are active in these particular pEJ-transfected cells, while all copies of the transfected pEC gene in the other cells might be active. In such a case, the comparable levels of protein and RNA observed would not accurately reflect the instrinsic transcriptional activities of the two genes.

However, one point emerges with clarity from these experiments: a certain level of EJ-specified p21 induces transformation, while a comparable if not higher level of the proto-oncogene-specified p21 has no effect on cellular phenotype. Since the p21 proteins are the only apparent gene products encoded by these genes, we conclude that the difference in function between the EJ oncogene and the proto-oncogene must derive from structural alterations in the p21 protein. Conversely, regulatory alterations do not seem critical to the transforming activity of the oncogene.

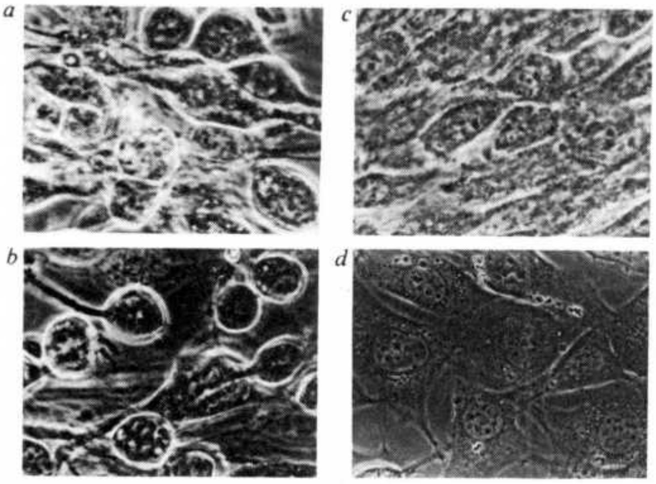

Fig. 3 Photographs of cell lines transfected with pEJ or pEC through a phase contrast microscope (×300). Cell lines were derived as described in Fig. 2 legend. Cell lines transfected with pEJ are shown at confluence (EJ/Gpt-2, *a*) and subconfluence (EJ/Gpt-3, *b*). Cell lines transfected with pEC are also shown at confluence (EC/Gpt-5, *c*) and subconfluence (EC/Gpt-1, *d*).

Detection of differences in the size of the oncogene product

In view of the above conclusion, new importance was attached to the previously detected slight variations in migration rates of the p21 proteins from different cells (Figs 1*b* and 2*b*). We therefore re-analysed the p21 proteins after shorter periods of metabolic labelling, which we expected would highlight these differences more clearly. Data shown in Fig. 4*a* indicate that the pEJ and pEC transfectants each exhibit two bands of p21. Kinetic labelling experiments indicate that in each case the more slowly migrating band behaves as a kinetic precursor to the more rapidly migrating band (manuscript in preparation). Comparable data on the p21 protein of v-Ha-*ras* previously indicated that the higher band underwent post-translational cleavage to yield its lower, more rapidly migrating partner[28].

Further examination of Fig. 4*a* reveals that the higher molecular weight p21 protein of the pEJ transfectant migrates slower than the higher molecular weight protein of the pEC transfectant and also that the lower molecular weight p21 of the pEJ transfectant migrates slower than the lower molecular weight p21 of the pEC transfectant. The relative migration rates of the these proteins, and the relationships we impute to them from kinetic data and previously published experiments[28] are indicated schematically in Fig. 4*a*.

As none of the p21 proteins seems to be phosphorylated to any extent (data not shown), the differences in migration between the pEC and pEJ proteins are most readily attributed to alterations in the number of amino acids or to changes in conformation. The data and the schematization of Fig. 4*a* may provide an explanation for the complexity of p21 proteins seen in normal and transformed bladder cells (Fig. 1*b*): the normal cells exhibit two bands, reflective of the expression of a proto-oncogene; the carcinoma cells appear to exhibit four bands, two being specified by the oncogene of these cells, and two by the normal, proto-oncogene of the other, homologous chromosome.

Genetic mapping of the functionally altered region of the oncogene

To determine whether the observed physical difference between p21 proteins reflected a functionally important change involved in the process of transformation, we set out to supplement these results with an independent series of experiments designed to localize genetically the regions of the oncogene that specify the altered migration rate of the protein and the change in gene function. These experiments depended on *in vitro* homologous recombination between clones of the two genes. The experimental design was to excise a restriction fragment out of the oncogene and use it to replace the homologous piece of the proto-oncogene. At the same time, the reciprocal construction was carried out by splicing the fragment of the proto-oncogene into the oncogene. These recombinant constructs were then tested for transforming ability in the transfection assay. We looked for the ability of a fragment of the oncogene to impart transforming activity when placed in the midst of a proto-oncogene clone, and conversely, in the reciprocal recombination, for the loss of activity when the corresponding proto-oncogene fragment was inserted into the oncogene clone. The constructions are shown schematically in Fig. 5. Following *in vitro* ligation, the recombinant clones were amplified and mapped to confirm their construction. Only then were they transfected to NIH 3T3 monolayers.

It was vital that we should be able to verify that the transforming clones were indeed chimaeras of mixed pEJ and pEC origin, rather than contaminants of one origin or the other. This was

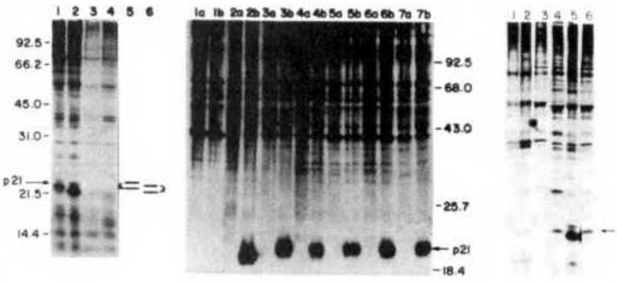

Fig. 4 Comparison of the mobility of p21 proteins encoded by the EJ bladder carcinoma transforming gene and its normal homologue. *a*, Immunoprecipitations from cells transfected with pEJ or pEC. Cells were metabolically labelled with ^{35}S-methionine for 3 h; cell lysates were prepared, immunoprecipitated and analysed as described in Fig. 1 legend. Cell lines were derived as described in Fig. 2 legend. Lysates from the cell line EJ/Gpt-3 (lanes 1, 3) and from the cell line EC/Gpt-1 (lanes 2, 4) were precipitated with the monoclonal anti-p21 antiserum Y13-238 (lanes 1, 2) or with non-immune serum (lanes 3, 4). Schematic diagrams (lane 5, EJ/Gpt-3; lane 6, EC/Gpt-1) show both the relative positions of the detected p21 bands and the relationships of those bands (arrows) based on kinetic data (not shown) and previously published experiments[28]. *b*, Analysis of the migration of p21 proteins immunoprecipitated from cells transfected with *in vitro* recombinants of the EJ oncogene (pEJ) and its homologous proto-oncogene (pEC). NIH 3T3 cells transformed with the EJ bladder tumour oncogene, its normal proto-oncogene, or recombinants between the two genes were first biologically cloned in 0.35% agar and then metabolically labelled with ^{35}S-methionine for 18 h. Lysates were prepared and immunoprecipitated (5×10^6 c.p.m. of TCA-precipitable counts) by a monoclonal antibody that detects the p21 encoded by Ha-MuSV but not the p21 encoded by Ki-MuSV (Y13-172)[25,32]. Dissolved immunoprecipitates were then resolved by electrophoresis in a 12% SDS-polyacrylamide gel. Lanes 1a–7a, no antibody; lanes 1b–7b, anti-Harvey p21 monoclonal antibody. Cell lysates were from: NIH 3T3 cells (lane 1); cells transformed with the proto-oncogene (the 3-kb *Sac*I fragment of the pEC proto-oncogene that has been activated by fusion with a retrovirus LTR transcriptional promoter described in ref. 35) (lane 2); cells transformed with the EJ oncogene (6.6-kb fragment in pBR) (lane 3); clone 511-74, transformed with the ligation of proto-oncogene 1-kb *Sac*I fragment to EJ oncogene 3-kb *Sac*I fragment (construction b2, Fig. 5) (lane 4); cells transformed with ligations of a fragment of the EJ oncogene extending from the *Xho*I site to the second *Bst*EII site to a clone of the proto-oncogene from which the homologous fragment had been removed (construction e2, Fig. 5) (lanes 5 and 6); and cells transformed with the ligation of the EJ oncogene to the left of the *Kpn*I site to the proto-oncogene to the right of this site (construction a2, Fig. 5) (lane 7). *c*, Analysis of the migration of the p21 proteins immunoprecipitated from cells transfected with the *in vitro* recombinant in which the 350-bp *Xma*I–*Kpn*I fragment of the pEJ oncogene clone was inserted into the corresponding region of the pEC proto-oncogene clone. Cells were metabolically labelled with ^{35}S-methionine for 3 h; cell lysates were prepared, immunoprecipitated and analysed as described in Fig. 1 legend. Cell lines EC/Gpt-1 and EJ/Gpt-3 were derived as described in Fig. 2 legend. The *in vitro* recombinant was constructed as described in Fig. 5 legend, part h2. The resulting construct was transfected as described in Fig. 2 legend and a transformed focus was picked and expanded. Immunoprecipitations were performed with non-immune serum (lanes 1–3) or with the monoclonal antiserum (Y13-238) which precipitates the Ha-MuSV p21 (lanes 4–6). Cell lysates were prepared from EJ/Gpt-3 (lanes 1 and 4); EC/Gpt-1 (lanes 2 and 5); and the *Xma*I–*Kpn*I recombinant transfected cell line (lanes 3 and 6).

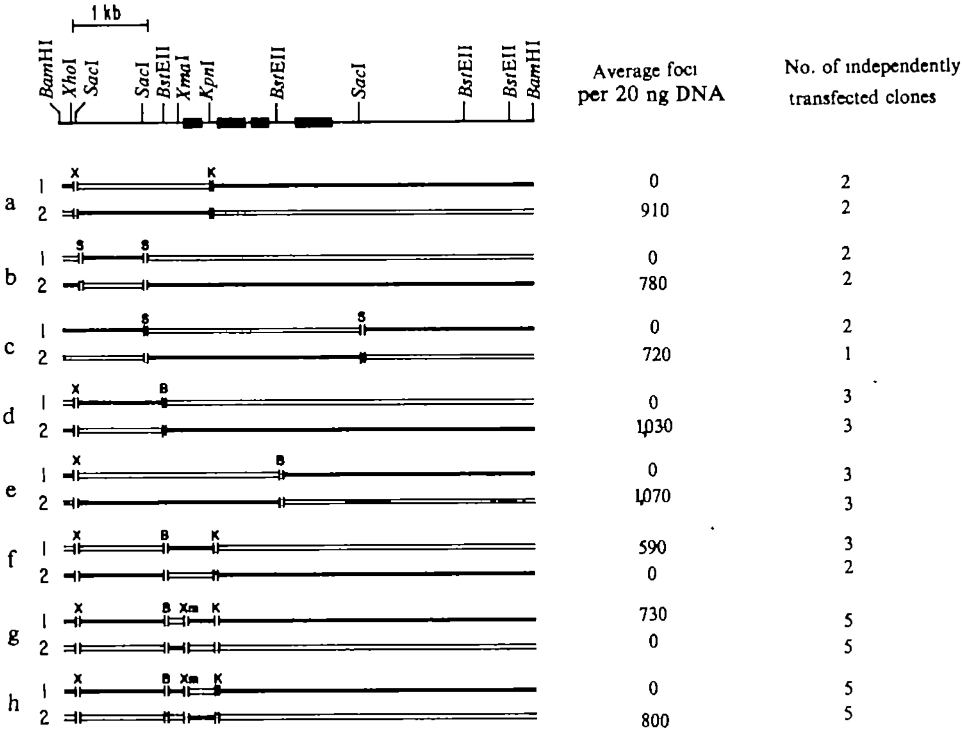

Fig. 5 Transfection data and structures of *in vitro* genetic recombinants between the molecular clones of the EJ transforming gene (pEJ) and its normal cellular homologue (pEC). The restriction map shows the cleavage sites for various enzymes within the 6.6-kb *Bam*HI insert in pBR322. All sites specific for the enzymes are shown except for *Xma*I which cuts in several other places which have not been well characterized. The site shown is the only *Xma*I site between the first *Bst*EII site and the *Kpn*I site The solid boxes on the map show the locations of coding exons pEJ/pEC chimaeras are shown, with segments derived from pEJ shown as solid bars and segments from pEC shown as open bars. pEJ and pEC were cleaved with the indicated enzymes either to completion or in a partial digest as required to obtain each indicated fragment The products were separated by electrophoresis through 1.2% agarose and eluted by melting in NaI and adsorbing to glass beads. The fragment containing pBR322 was then treated with calf intestinal phosphatase. The indicated fragments were joined either with the enzyme T4 DNA ligase or in a mock ligation without enzyme. Constructs a–e were made in bimolecular ligations. Constructs in f were made by mixing the three fragments simultaneously and in g and h by mixing the four fragments simultaneously The ligation mixtures were directly transformed into the HB101 strain of *Escherichia coli*. Only when colonies from mock ligations were less than 2% of the ligations were colonies analysed for the presence of clones with appropriate restriction maps; 20 ng of each clone were transfected to NIH 3T3 cells as described in Fig. 2 legend and then carried without selection until foci were visualized in 10–14 days. Results of the transfections are shown in the first column. The second column shows the number of independent bacterial colonies screened and then transfected into NIH 3T3 cells.

done in three ways. In the simpler constructions, involving ligations of two fragments at a time, we verified results obtained with amplified recombinant clones by directly transfecting the unamplified products of ligation reactions and of mock ligations containing isolated fragments not treated with the ligase. A second confirmation depended on the fact that the plasmids pEJ and pEC contained their respective cellular genes inserted in the pBR322 plasmid vector in opposite orientations. Thus, we could learn the origin of one parent of a recombinant by diagnostic restriction digests of the flanking plasmid regions. Since contaminating pEC could itself not give a false positive result (as it is not transforming), any active clone carrying proto-oncogene flanking sequences must have arisen as a consequence of acquisition of portions of the transforming gene. Finally, we confirmed all of our results with several independent clones obtained from a ligation reaction. The products of a ligation were used only if mock ligation of the same fragments gave no more than 2% of the bacterial colonies seen on use of the DNA ligase.

The results of transfecting the recombinant clones are summarized in Fig. 5. As can be seen, we ultimately identified a genetic region 350 nucleotides long which, when transferred from the oncogene to a corresponding region in the proto-oncogene, is able to impart activity to the latter. This region extends from the first *Xma*I endonuclease site to the *Kpn*I site (Fig. 5); 55% of this region consists of the first coding exon, 10% is 5′ to the coding region and 35% is part of the first intron.

Detection of differences in size of the gene product from the *in vitro* recombinants

In experiments described above, we had identified a difference in the migration of the p21 protein encoded by the oncogene and the proto-oncogene. Having now determined a short region of the gene which contained the transforming lesion, it became important to ascertain whether the region also contains the specificity for the altered protein size. We therefore carried out immunoprecipitations on cells transfected with the products of the *in vitro* recombinants. As seen in Fig. 4*b*, the protein brought down in lysates from *Xho*I–second *Bst*II site, *Sac*I–*Sac*I, and *Xho*I–*Kpn*I recombination transfectants all co-migrate with the EJ protein and have a mobility which differs from that of the EC protein. Similarly, Fig. 4*c* demonstrates this result for the *Xma*I–*Kpn*I recombinants. The lysates analysed in panel 4*b* were from cultures that were labelled for 18 h, resulting in high levels of label in the lower molecular weight forms of the p21, and undetectable amounts of label in the kinetically unstable higher molecular weight forms. We conclude that the phenotypes of oncogenic transformation and altered electrophoretic migration co-segregate, and the two are encoded by the same 350-bp segment of DNA. As indicated below, the altered migration rate is probably a reflection of a functionally important alteration of the protein.

Nucleotide sequence of the transforming lesion

Having identified a short fragment of biological significance, we then sequenced this region of the oncogene and the proto-oncogene. Figure 6 compares the sequences of the two genes in this segment obtained by sequencing both strands. The DNA sequence described in the figure was obtained by the forward and backward dideoxy DNA sequencing technique of Seif *et al.*[29] and by the chemical procedure of Maxam and Gilbert[30].

The difference between the two segments is in the p21 encoding region of the first known exon, specificially 60 nucleotides from the *Xma*I cleavage site. It occurs in a triplet that encodes glycine in the normal rat (M. Ruta *et al.*, unpublished data) and human c-Ha-*ras* genes. The sequence observed in the EJ oncogene causes conversion to a valine. As we discuss below, we believe that this alteration is responsible for the alteration in function of the p21 protein, and for the oncogenic activation of the c-Ha-*ras* gene that occurred in the EJ bladder carcinoma.

Alteration of a diagnostic restriction endonuclease cleavage site

A second independent consequence of the single base change was the alteration in the cleavage sites of two different site-specific endonucleases. The sequence GCCGGC occurs in the proto-oncogene, and thus represents the recognition site of the endonuclease *Nae*I. It also contains the CCGG recognition site of the endonuclease *Hpa*I. Both of these are changed in the oncogene, the equivalent sequence of which reads GCCGTC. The *Nae*I endonuclease is the more useful of the two since it cleaves DNA less frequently, and we used it to independently verify the two sequences. As expected, the pEC clone exhibited one more cleavage site in its inserts than its pEJ counterpart (data not shown). Perhaps more useful was the retrospective verification of the *in vitro* recombinant clones. The allele specifying transformation and abnormal p21 migration was seen to precisely co-segregate with the allele disallowing *Nae*I cleavage at this site (data not shown).

Discussion

Use of gene transfer and molecular cloning had previously made it possible to localize an important lesion of the EJ bladder carcinoma genome to a 6.6-kb DNA segment constituting only 10^{-6} of the genetic content of the tumour cell. The segment appears to contain the entire transforming activity previously associated with the whole tumour cell DNA. This oncogene is an important, but perhaps not the sole, determinant of the transformed phenotype of these carcinoma cells. Sequence hybridization showed that this oncogene was related in structure to a similar sequence existing in the normal cellular genome[12–15]. It appeared that the oncogene arose from the activation of these normal sequences, and that this activation occurred during the process of carcinogenesis which led to formation of the original EJ carcinoma. Experiments described in this report were designed to resolve and define the critical differences that distinguish the oncogene from its proto-oncogene precursor.

It was apparent that a definition of these differences could not be achieved by simple comparison of the sequences of the two genes. As the oncogene and its normal proto-oncogene counterpart sequence were derived from the DNAs of separate individuals, most sequence differences might reflect naturally occurring, silent polymorphisms at this locus. Other sequence differences could be consequences of mutational insults suffered during carcinogenesis, which were silent functionally and thus of no importance to the activation process. These considerations caused us to undertake the *in vitro* recombination experiments in order to narrow the area of functional importance to a segment so small that it would probably contain few if any silent mutations. Only after narrowing the functional segment to 350 bp did we initiate DNA sequence studies.

The sequence difference that we observed in this region could have only one consequence for the structure of the p21 protein—a simple amino acid substitution leading to the replacement of a glycine by a valine residue. This substitution appears to represent the critical agent of the conversion of the proto-oncogene into an active oncogene.

```
                                                   XmaI
                                                    ↓
                                                   CCCGGG CCGCAGGCCC TTGAGGAGCG

                                            gly
met thr glu tyr lys leu val val val gly ala GGC gly val gly lys ser ala leu thr
ATG ACG GAA TAT AAG CTG GTG GTG GTG GGC GCC GTC GGT GTG GGC AAG AGT GCG CTG ACC
                                            val

                                                                    splice
ile gln leu ile gln asn his phe val asp glu tyr asp pro thr ile glu ↓
ATC CAG CTG ATC CAG AAC CAT TTT GTG GAC GAA TAC GAC CCC ACT ATA GAG  GTGAGCCTGC

GCCGCCGTCC AGGTGCCAGC AGCTGCTGCG GCCGAGCCCA GGACACAGCC AGGATAGGGC TGGCTGCAGC

CCCTGGTCCC CTGCATGGTG CTGTGGCCCT GTCTCCTGCT TCCTCTAGAC GAGGGGAGTC CCTCGTCTCA

GCACCCCAGG AGAGGAGGGC GCATGAGGGG CATGAGAGGT ACC
                                             ↑
                                            KpnI
```

Fig. 6 Comparison of the DNA sequence of the molecular clone of the EJ transforming gene (pEJ) and its non-transforming cellular homologue (pEC). Sequence data between the restriction enzyme sites *Xma*I and *Kpn*I are shown (see Fig. 5). The coding DNA strand is printed as is the inferred amino acid sequence. For coding regions where pEJ and pEC differ, both sequences are indicated. Sequences were determined by the forward and backward dideoxy DNA sequencing technique of Seif *et al.*[29] and by the chemical procedure of Maxam and Gilbert[30].

Such a simple substitution might not be expected to effect a profound change in the function of the p21 protein. However, several considerations seem to confer importance on the structural alteration. The first stems from comparison of this domain of p21 encoded by the human c-Ha-*ras*-1 gene, the rat c-Ha-*ras*-1 gene (Ruta *et al.*, unpublished results) and the v-Ha-*ras* oncogene of Harvey sarcoma virus[31]. The 37-residue long amino acid sequences encoded by the first exons of the two cellular genes are identical, indicating strict evolutionary conservation of this region. However, analysis of the Harvey sarcoma virus oncogene reveals that it deviates from its direct rat cellular antecedent in only one position in this domain, a glycine to arginine conversion at precisely the same residue that is altered in the EJ oncogene. Thus, we speculate that alteration of this critical residue was important both in the activation of the v-Ha-*ras* gene from its rat cellular precursor, and in the activation of the EJ bladder oncogene from its normal human counterpart. Moreover, a similar alteration may have been significant in the oncogenic activation of another member of the *ras* gene family, the v-Ki-*ras* gene of Kirsten murine sarcoma virus. The Kirsten transforming gene is closely related to the v-Ha-*ras* gene[32]. The only difference between the p21 of v-Ki-*ras* and that of v-Ha-*ras* in the first 36 amino acids is at position 12, where the residue in Kirsten is serine[33], again at precisely the same site as altered in the EJ and Harvey sarcoma virus encoded p21s. While sequence information is not available on the cellular homologue of v-Ki-*ras*, we speculate that a conversion from a glycine to a new amino acid residue at position 12 may also have been involved in the activation of this *ras* oncogene.

A second consideration stems from examination of the specific amino acid changes observed in these cases. In both instances, glycine is replaced by an amino acid having a relatively bulky side chain. Glycine is an anomaly among the 20 amino acids, because it lacks a side chain. Thus, it is able to participate in extremes of bending and folding of the polypeptide backbone and is the strongest breaker of α-helices[34]. Thus, replacements of glycine by valine or arginine represent abrupt changes in the local stereochemistry of a protein.

We speculate that the loss of glycine at residue 12 causes a significant change in an essential domain of the p21 protein. One consequence of this change may be a conformational shift of the protein, leading in turn to the aberrant electrophoretic migration or processing of the p21 proteins. A second, more important consequence is a profound effect on the function of the p21 protein. This alteration probably affects interaction of the p21 with cellular targets. A precedent exists for other single amino acid changes having profound effects on cellular and organismic physiology. The most well known of these is the sickle-cell syndrome, in which a glutamine to valine conversion affects the solubility of haemoglobin within erythrocytes.

The importance we attach to alteration of protein structure would seem to contradict a series of experiments of recent years that indicate up-regulation as the pivotal event in carcinogenesis. These experiments include the activation of the *myc* proto-oncogene occurring during leukaemogenesis of avian retroviruses[35,36]; and the demonstration that *in vitro* fusion of a retroviral long terminal repeat (LTR) promoter and a cellular proto-oncogene results in an actively transforming gene[20,37,38]. These latter experiments are particularly germane, since some of them demonstrate activation of clones of the rat and human c-Ha-*ras* proto-oncogenes[20,38]. It is unlikely that the protein-encoding sequences of these c-Ha-*ras* genes have undergone any structural changes during construction of these viral–cellular chimaeras. Rather, it appears that the only essential difference between the proto-oncogenes and their LTR-activated counterparts lies in rates of expression. This means that the *ras* proto-oncogene can be activated by a second independent mechanism, in principle as effective in creating an oncogene as the one described here.

We point out that oncogenes of other tumours have also been traced to *ras* genes. Specifically, colon and lung carcinomas (ref. 15 and M. McCoy, J. J. Toole, E.H.C. and R.A.W., in preparation) as well as fibroblastic tumours (L. Parada and R.A.W., unpublished results) have been found to carry oncogenes derived from activation of cellular Ki-*ras* genes. As discussed above, the N-terminal regions of the Ha-*ras* and Ki-*ras* proteins are extremely similar, and the Ki-*ras* proteins of many of these tumours also exhibit aberrant electrophoretic migration rates (manuscript in preparation). We predict that activation of many of these other oncogenes will depend on structural alterations very similar to those reported here.

Most amino acid sequence alterations are either neutral or deleterious to protein function. Few are able to actively potentiate the normal functions of a protein. We suggest that only a small number of sites on the p21 protein can be altered in a fashion leading to oncogenic activation. Most mutations will affect other residues whose alteration will be unproductive for oncogenic conversion. The target for oncogenic conversion may therefore be exceedingly small, and may even be confined to the Gly-12 codon. In such a case, the restriction site for *NaeI*, which totally spans that codon, may provide an ideal diagnostic tool for detecting a critical change in the genome.

The present data suggest that the alteration of one nucleotide in one bladder cell leads to the creation of an activated oncogene. There are three possible point mutations at this position, and it is perhaps not coincidental that the G-T transversion observed here is precisely that mutation favoured by many suspected bladder carcinogens[39]. The oncogene resulting from this mutation was probably an important determinant in the subsequent outgrowth of a lethal neoplasm. The point mutation implicated as a central event in this oncogenic transformation represents the first demonstration of a lesion in cellular DNA whose occurrence is directly related to the carcinogenic process.

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A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene

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The genetic change that leads to the activation of the oncogene in T24 human bladder carcinoma cells is shown to be a single point mutation of guanosine into thymidine. This substitution results in the incorporation of valine instead of glycine as the twelfth amino acid residue of the T24 oncogene-encoded p21 protein. Thus, a single amino acid substitution appears to be sufficient to confer transforming properties on the gene product of the T24 human bladder carcinoma oncogene.

DNA-MEDIATED gene transfer techniques have made it possible to identify the presence of dominant transforming genes (oncogenes) in a variety of human tumours (for review see ref. 1). Although only a small number of human tumour DNAs have been shown to be capable of transforming normal cells in transfection assays, oncogenes have been detected in tumours representative of each of the major forms of human cancer[2–8]. To date, more than 10 different human oncogenes have been identified. One of them, present in T24 and EJ bladder carcinoma cell lines, has been isolated by molecular cloning techniques[8–10]. Preliminary characterization of this oncogene has revealed that it is small (less than 4.6 kilobase, kb), and that it has not undergone major genetic rearrangements[9–11]. Comparative analysis of this bladder carcinoma oncogene with retroviral transforming (*onc*) genes has revealed that an internal fragment of the T24 oncogene is closely related to the *onc* genes of

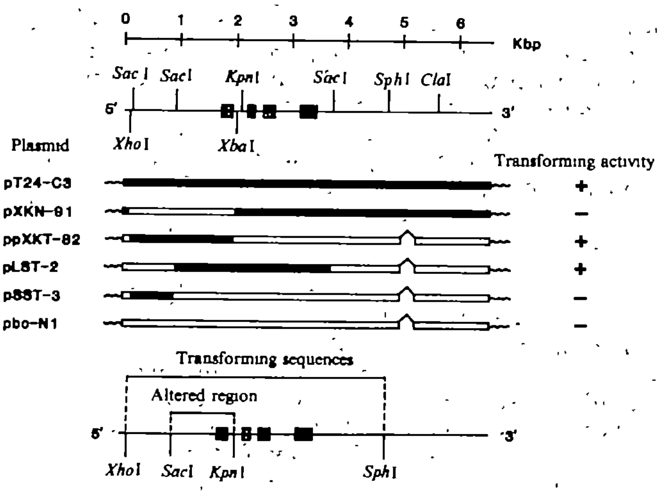

Fig. 1 Location of the region of the c-*has*/*bas*-1 gene that underwent the genetic alterations that led to the activation of the T24 oncogene. Hybrid plasmids containing sequences derived from pT24-C3 (■) and pbc-N1 (□) were tested for their biological activity. pT24-C3, pXKT-82 and pLST-2 transformed NIH 3T3 cells in transfection assays[8], with specific transforming activities of 5×10^4, 1×10^4 and 0.6×10^4 ffu pmol^{-1}, respectively. In contrast, pbc-N1, pXKN-91 and pSST-3 exhibited no transforming activity. The hatched boxes located within the restricted endonuclease map shown in the top of the figure represent the predicted exons of the T24 oncogene and its normal homologue, the c-*has*/*bas*-1 gene. Deletions (/\), and plasmid sequences (〰), are also indicated in the diagram. See text for definition of the regions encompassing the transforming sequences and the activated domain of the T24 oncogene.

Harvey and BALB murine sarcoma viruses (designated v-*has* and v-*bas*, respectively)[11–13]. Characterization of human DNA sequences homologous to v-*has* and v-*bas* has demonstrated that the normal c-*has*/c-*bas*-1 gene is in fact an allele of the T24 oncogene[11–14]. Moreover, we found that these genes, despite their different biological properties, were indistinguishable by both heteroduplex and restriction enzyme analysis[11–13]. In an effort to understand the molecular events involved in human carcinogenesis, studies were undertaken to determine the number and nature of the genetic alterations that have resulted in the activation of the T24 human bladder carcinoma oncogene.

A domain within the T24 oncogene responsible for its activation

Previously, we have reported that a plasmid (pT24-C3) known to contain a 6.6-kb *Bam*HI fragment of T24 cellular DNA was capable of transforming NIH 3T3 mouse cells with a specific activity of 5×10^4 focus-forming units (ffu) per pmol. A restriction endonuclease map of this DNA fragment is depicted in Fig. 1. The transforming activity of this molecule was localized to a region within the 4.6-kb *Xho*I–*Sph*I DNA fragment based on the observations that cleavage with these two restriction endonucleases did not affect the transforming activity of pT24-C3 (ref. 11). To establish the region of the normal human c-*has*/*bas*-1 gene that underwent the genetic alterations leading to its malignant activation in T24 bladder carcinoma cells, we constructed a series of plasmids in which fragments of the T24 transforming gene were substituted with the homologous normal sequences of c-*has*/*bas*-1. In reciprocal experiments, regions of the normal c-*has*/*bas*-1 gene were replaced by the corresponding domains of its transforming allele. Figure 1 summarizes the plasmids constructed in the present studies as well as their respective biological activities. Substitution in pT24-C3 of a DNA fragment located between the *Xho*I and *Kpn*I cleavage sites by normal sequences, completely abolished its transforming activity. In contrast, replacement of the normal *Xho*I–*Kpn*I region of pbc-N1, a plasmid that contains the 6.4-kb *Bam*HI DNA clone of the normal human c-*has*/*bas*-1 gene, by the corresponding domain of pT24-C3, generated a plasmid (pXKT-82) that transformed NIH 3T3 mouse cells with an efficiency of 10^4 ffu pmol^{-1}.

Two additional plasmids were generated by replacing the internal 0.7-kb and 3.0-kb *Sac*I DNA fragments of pbc-N1 by the corresponding regions of the T24 oncogene. As shown in Fig. 1, only the latter plasmid (pLST-2) was able to induce malignant transformation on transfection to NIH 3T3 cells. These results, taken together, establish that the genetic alterations that activated the T24 oncogene are located within the 0.9-kb *Sac*I–*Kpn*I DNA fragment of pT24-C3 DNA.

```
  Sac I
    ▼                                         50                                            100
GAGCTCCTCTGTCTTCTCCAGCTTTCTGTGGCTGAAAGATGCCCCCGGTTCCCCGCCGGGGGTGCGGGGCGCTGCCCGGGTCTGCCCTCCCCTCGGCGGC

                                             150                                            200
GCCTAGTACGCAGTAGGCGCTCAGCAAATACTTGTCGGAGGCACCAGCGCCGCGGGGCCTGCAGGCTGGCACTAGCCTGCCCGGGCACGCCGTGGCGCGC

                                             250                                            300
TCCGCCGTGGCCAGACCTGTTCTGGAGGACGGTAACCTCAGCCCTCGGGCGCCTCCCTTTAGCCTTTCTGCCGACCCAGCAGCTTCTAATTTGGGTGCGT

                                             350                                            400
GGTTGAGAGCGCTCAGCTGTCAGCCCTGCCTTTGAGGGCTGGGTCCCTTTTCCCATCACTGGGTCATTAAGAGCAAGTGGGGGCGAGGCGACAGCCCTCC

              Pvu II
                 ▼                           450                                            500
CGCACGCTGGGTTGCAGCTGCACAGGTAGGCACGCTGCAGTCCTTGCTGCCTGGCGTTGGGGCCCAGGGACCGCTGTGGGTTTGCCCTTCAGATGGCCCT

                                             550                                            600
GCCAGCAGCTGCCCTGTGGGGCCTGGGGCTGGGCCTGGGCCTGGCTGAGCAGGGCCCTCCTTGGCAGGTGGGGCAGGAGACCCTGTAGGAGGACCCCGGG

                                                       650  G
CCGCAGGCCCTTGAGGAGCG ATG ACG GAA TAT AAG CTG GTG GTG GTG GGC GCC GTC GGT GTG GGC AAG AGT GCG CTG ACC
         ...         Met Thr Glu Tyr Lys Leu Val Val Val Gly Ala Val Gly Val Gly Lys Ser Ala Leu Thr

   Pvu II                                                          Splice
      ▼             700                                           ▼⇨                 750
ATC CAG CTG ATC CAG AAC CAT TTT GTG GAC GAA TAC GAC CCC ACT ATA GAG GTGAGCCTGGCGCCGCCGTCCAGGTGCCAGCA
Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr Asp Pro Thr Ile Glu

                                                                                          Xba I
                                   800                                             850       ▼
GCTGCTGCGGGCGAGCCCAGGACACAGCCAGGATAGGGCTGGCTGCAGCCCCTGGTCCCCTGCATGGTGCTGTGGCCCTGTCTCCTGCTTCCTCTAGAGG

                                                          Kpn I
                                   900                      ▼
AGGGGAGTCCCTCGTCTCAGCACCCCAGGAGAGGAGGGGGCATGAGGGGCATGAGAGGTACCAGGGA
                                          ...     ...
```

Fig. 2 Comparative sequence analysis of the 0.93-kb *Sac*I–*Kpn*I DNA fragment of the T24 oncogene and its normal human homologue c-*has*/*bas*-1. The upper line shows the sequence of the T24 oncogene proceeding in the 5′ to 3′ direction with respect to the polarity of the closely related v-*has* and v-*bas* retroviral mRNAs. The corresponding sequence of the 0.93-kb *Sac*I–*Kpn*I DNA fragment of c-*has*/*bas*-1 has also been determined and found to be identical to that of the T24 oncogene with the exception of the nucleotide located at position 653, where we detected a G instead of a T. These nucleotide chains result in the incorporation of glycine, instead of valine, at position 12 in the p21 protein coded for by the c-*has*/c-*bas*-1 gene. Termination codons are indicated (···). The nucleotide sequence analysis was performed according to the procedures of Maxam and Gilbert[23].

Fig. 3 Predicted amino acid sequence of the first exon of the normal human c-*has*/*bas*-1 gene and its transforming allele, the T24 oncogene. Comparison with the first 37 amino acids predicted for the p21 proteins coded for by Harvey-MSV[15] and Kirsten-MSV[18]. The letter code for the amino acids is: A, Ala; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; and Y, Tyr.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Human c-bas/ras^H	M	T	E	Y	K	L	V	V	V	G	A	G	G	V	G	K	S	A	L	T	I	Q	L	I	Q	N	H	F	V	D	E	Y	D	P	T	I	E
T24 oncogene	M	T	E	Y	K	L	V	V	V	G	A	V	G	V	G	K	S	A	L	T	I	Q	L	I	Q	N	H	F	V	D	E	Y	D	P	T	I	E
Harvey-MSV	M	T	E	Y	K	L	V	V	V	G	A	R	G	V	G	K	S	A	L	T	I	Q	L	I	Q	N	H	F	V	D	E	Y	D	P	T	I	E
Kirsten-MSV	M	T	E	Y	K	L	V	V	V	G	A	S	G	V	G	K	S	A	L	T	I	Q	L	I	Q	N	H	F	V	D	E	Y	D	P	T	I	Q

Comparative sequence analysis of the 0.9-kb *Sac*I–*Kpn*I DNA fragments of pT24-C3 and pbc-N1

The nucleotide sequence of the 0.9-kb *Sac*I–*Kpn*I DNA fragments of the T24 oncogene and its normal homologue was next established. To our surprise there was only a single (nucleotide) change, located at position 655 from the *Sac*I cleavage site. Figure 2 shows that the guanosine residue present in the normal c-*has*/*bas*-1 gene has been changed into a thymidine residue in the T24 oncogene. Thus the activation of the c-*bas*/*ras*-1 gene in T24 human bladder carcinoma cells was caused by a single point mutation.

The mutated guanosine residue was found to be part of the hexanucleotide sequence GCCGGC, which is specifically recognized by the restriction endonuclease *Nae*I. Thus, we used this enzyme to verify the results obtained by nucleotide sequence analysis. We observed that *Nae*I cleaved the 6.4-kb *Bam*HI insert of pbc-N1 five times, yielding four internal DNA fragments, of sizes 0.2, 0.4, 0.6 and 0.9 kb. In contrast, digestion of pT24-C3 with *Nae*I yielded only three internal fragments, of sizes 0.2, 0.6 and 1.3 kb (data not shown). The loss of the *Nae*I cleavage site within the 1.3-kb internal fragment confirms the nucleotide sequence shown in Fig. 2.

Heteroduplex analysis of the T24 oncogene and its normal allele with v-*has* and v-*bas* had indicated that the putative coding sequences of the human genes are distributed among four exons, of which only the first one lies within the 0.9-kb *Sac*I–*Kpn*I fragment[11,14]. We have sequenced the entire coding region of the T24 oncogene and identified the four exons by comparison of these sequences with those of v-*has*[15] and v-*bas*[16]. This sequence analysis demonstrated that the first exon ends at position 731 from the *Sac*I cleavage site (Fig. 2). At this position, we detected the consensus donor splice sequence AGAGG[17]. Thus, in agreement with previous heteroduplex analysis and restriction enzyme data[11,14], the first exon of the T24 oncogene contained the coding information for 37 amino acids extending from position 621 to 731. These observations located the mutated G residue at the second base of a triplet coding for glycine, the predicted twelfth amino acid residue of the normal human p21 protein. Substitution of this guanosine residue for thymidine results in the change of the glycine-coding triplet GGC to GTC, which codes for valine. Thus, a single amino acid substitution (Gly→Val) seems to be sufficient to confer transforming properties to the gene product of the T24 human bladder carcinoma oncogene.

A common genetic alteration in human and retroviral transforming genes

The amino acid sequences of Harvey and BALB-MSV-encoded p21 proteins have been identified by nucleotide sequence analysis of their corresponding *onc* genes, v-*has* and v-*bas*, respectively[15,16]. Comparison of the first 37 amino acids of the p21 protein coded for by the normal c-*has*/*bas*-1 human gene with their retroviral counterparts shows complete identity except at position 12, the same amino acid residue that is altered in the T24 oncogene (Fig. 3). Whereas arginine is present in Harvey-MSV p21[15], lysine was found in BALB-MSV p21[16]. Interestingly, the normal rat homologue of human c-*has*/*bas*-1, the gene that presumably recombined with Moloney-MuLV to generate Harvey-MSV, also possesses glycine at position 12 (M. Ruta, personal communication). These results suggest that mutations affecting the coding properties of the twelfth codon of normal c-*has*/*bas*-1 genes may be sufficient for the acquisition of malignant properties.

Recently, Tsuchida *et al.* have also reported the nucleotide sequence of v-*kis*, the *onc* gene of Kirsten-MSV[18]. When the first 37 amino acids of the deduced sequence of Kirsten p21 were compared with those of c-*has*/*bas*-1, only two changes were detected: Gly→Ser in position 12, and Glu→Gln in position 37. Thus, it is possible that changes in the glycine residue at position 12 may have also activated c-*kis* genes. This hypothesis would predict that another human transforming oncogene, present in a large variety of human carcinomas and known to contain the human c-*kis*-2 gene,[5,6,8,12,14,19] has been activated by the same mechanism that originated the T24 oncogene.

Activation of the human c-*has*/*bas*-1 gene: a qualitative or quantitative mechanism?

That each of the known p21 transforming proteins has a different amino acid at residue 12 suggests that their malignant properties are the result of the elimination of the glycine residue present in their normal counterparts, rather than due to a specific transition from one type of amino acid residue to another. Glycine residues are known to alter the α-helical structure of proteins. We compared the predicted secondary structures of the p21 proteins coded for by the T24 oncogene and its normal human homologue using the computerized method of Garnier *et al.*[20]. Such an analysis revealed that the N-terminal region of the c-*has*/*bas*-1-encoded p21 protein contains two α-helical regions of 10 and 13 amino acids joined by a hinge region of 7 amino acids that provides flexibility for folding. When the glycine at position 12 is replaced by valine in the transforming p21 protein, the size of the hinge region decreases to a stretch of two amino acids, resulting in an increased helical content of the N-terminal region of this protein. This change should result in the prominent projection of the N-terminal domain of transforming p21 proteins away from the central core of the molecule.

The biological significance of the structural alteration of human and retroviral p21 transforming proteins remains to be determined. Shih *et al.* have recently reported that the Harvey-MSV p21 protein is processed before its association with membranous structures[21]. Whether the presence of a glycine residue in position 12 confers on the normal cellular p21 protein a configuration that affects either its processing or its association with putative cellular targets remains to be determined. Whatever the mechanism might be, our findings indicate that the difference between the normal c-*has*/*bas*-1 and the T24 oncogene-encoded p21 proteins is of a qualitative nature.

However, it is also possible that quantitative alterations in the expression of the normal p21 protein induce malignant transformation. Chang *et al.* have shown that the normal c-*has*/*bas*-1 can transform NIH 3T3 mouse cells when this gene comes under the regulatory influence of a retroviral LTR[22]. In this case high levels of normal p21 protein can be detected in the transformed cells. Thus it is possible that increasing amounts of normal p21 protein may overcome the structural limitations imposed by the presence of a glycine residue at position 12. However, the possibility that over-expression of the normal c-*has*/*bas*-1 genes may induce morphological transformation by a different mechanism than that of their transforming counterparts cannot be ruled out at present.

Implications

The present studies have located the genetic changes leading to the activation of the T24 bladder carcinoma oncogene within a 0.9-kb *Sac*I–*Kpn*I DNA fragment of the human c-*has*/*bas*-1 gene. This region contains some 5′ putative regulatory sequences, as well as the first exon (Fig. 2). Comparative sequence analysis of these 0.9-kb DNA fragments derived from the T24 oncogene and its normal counterpart revealed a single base pair difference: a point mutation (G→T) within the exon sequences that led to the substitution of a glycine residue in the normal c-*has*/*bas*-1 gene-encoded p21 protein to a valine residue in the corresponding translational product of the T24 oncogene. These results, taken together, indicate that a single point mutation was responsible for the activation of this oncogene in T24 human bladder carcinoma cells.

These findings may have important practical applications. The mutated guanosine residue is part of a sequence (GCCGGC) recognized by the restriction endonucleases *Nae*I (GCCGGC), *Hpa*II (CCGG) and *Msp*I (CCGG). The G→T change observed in the T24 oncogene eliminated the cleavage site for these three enzymes. Neither *Nae*I nor *Hpa*II appear to cleave this sequence in human DNA, probably due to DNA methylation. However it is possible that *Msp*I, which recognizes the CCGG sequence whether methylated or not, could be used for the screening of human DNAs for the presence of T24-like oncogenes. Similarly, antibodies capable of specifically recognizing the altered domain of transforming p21 proteins may also be used to detect activated c-*has*/*bas* genes.

Demonstration that a single genetic change can activate a human transforming gene poses the dilemma as to how to relate these observations with the widely accepted multi-stage model for the development of human neoplasias. It is possible that activation of the T24 oncogenes may represent a late, irreversible step in oncogenesis. Support for this hypothesis comes from the fact that NIH 3T3 mouse cells, the cells used to identify this oncogene, are thought to be pre-neoplastic rather than normal cells. However, the possibility that this oncogene may have a role in the onset of certain human cancers cannot be excluded at present.

Development of biochemical and immunological reagents capable of specifically detecting the presence of the T24 bladder carcinoma oncogene (or its gene product) in both naturally and experimentally induced neoplasms should help to define the role of this dominant transforming gene in carcinogenesis.

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LETTERS TO NATURE

UV observations of TT Arietis and the magnetic rotator hypothesis

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TT Arietis, one of the brightest cataclysmic variables, $m_v \sim 10.2 \rightarrow 11.0$, is usually classed as a novalike variable, but after a recent dip in brightness to $m_v \sim 14.5$, Krautter *et al.*[1] suggested it to be a dwarf nova in an extended high state. Photometric light curves[2–4] generally show a sinusoidal light variation with a period of 0.1329 days and no eclipses[5]. Cowley *et al.*[6] have measured radial velocity curves and find a period of 0.13755 days. We have made[7] IR measurements of the system from which we deduce a distance for TT Ari of 100 ± 20 pc based on a colour change for the system from V to K of +0.4 while the system is faint to $V-K=-0.17$ when brighter. The red colour is caused by dilution of the primary 2.2-μm light by the light of the secondary, giving $m_K = 11.75^{+0.45}_{-0.25}$ for the secondary star; Bailey's method[8] then leads to the distance. The sinusoidal light variations have generally been interpreted as due to a hot spot on the edge of an accretion disk. Since good quality IUE spectra can be made with exposure times of about 15 min, we decided to observe as many IUE spectra as possible round the binary period with a view to studying the behaviour of the hot spot at UV wavelengths. We now report that the variations at these and X-ray wavelengths round the orbital cycle rule out a hot spot/accretion disk model for TT Ari. Instead we propose that it has a magnetized white dwarf primary rotating with the photometric period of 0.1329 days.

Four short and four long wavelength low dispersion spectra were obtained with the IUE satellite spectrometer on 9 January, 1982. Between each exposure the fine error sensor (FES) was used to measure the white light variation of TT Ari. The spectra taken nearest to maximum light, are shown in Fig. 1, together with an optical spectrum measured by M. J. Ward with the Anglo Australian Telescope. Although the spectra appear noisy, most of the features are repeatable from one spectrum to the next. The strongest features are: Lyα 1,216 e (geocoronal?); N V 1,238 a; Si IV 1,393 a; C IV 1,549 e (2.9); He II 1,640 a; and Mg II 2795 e (2.1) where 'a' denotes absorption, 'e' emission and the number in parentheses the variation in line strength (maximum/minimum). The variation of the emission lines roughly follows that of the continuum variation. Regions as free as possible of absorption or emission lines were used to measure the continuuum light variations. The continuum was averaged over a bandwidth of 50 Å. The only significant errors in the measurements are due to the photometric accuracy of the IUE cameras, namely ≤ ±10%.

Figure 2 shows the four point continuum light curves at 1,265, 1,700, 2,110, 2,510 and 3,010 Å, together with the nine point white light FES light curve. The amplitude of the white light curve corresponds to $m_v = 11.86 \rightarrow 12.36$; thus TT Ari was unusually faint. The solid curves are an attempt to fit a curve of the same shape as the white light curve but with variable amplitude through the UV points. The main difficulty in getting a good fit to the UV points is that the FES points represent ~1 min of integration and are thus prone to the flickering whereas the UV points result from spectra with 15-min exposure times. Whichever numbers are used for the amplitude of the variations the trend is clear; maximum amplitude being found for white light and the hard UV with a minimum variation in between at 3,010 Å.

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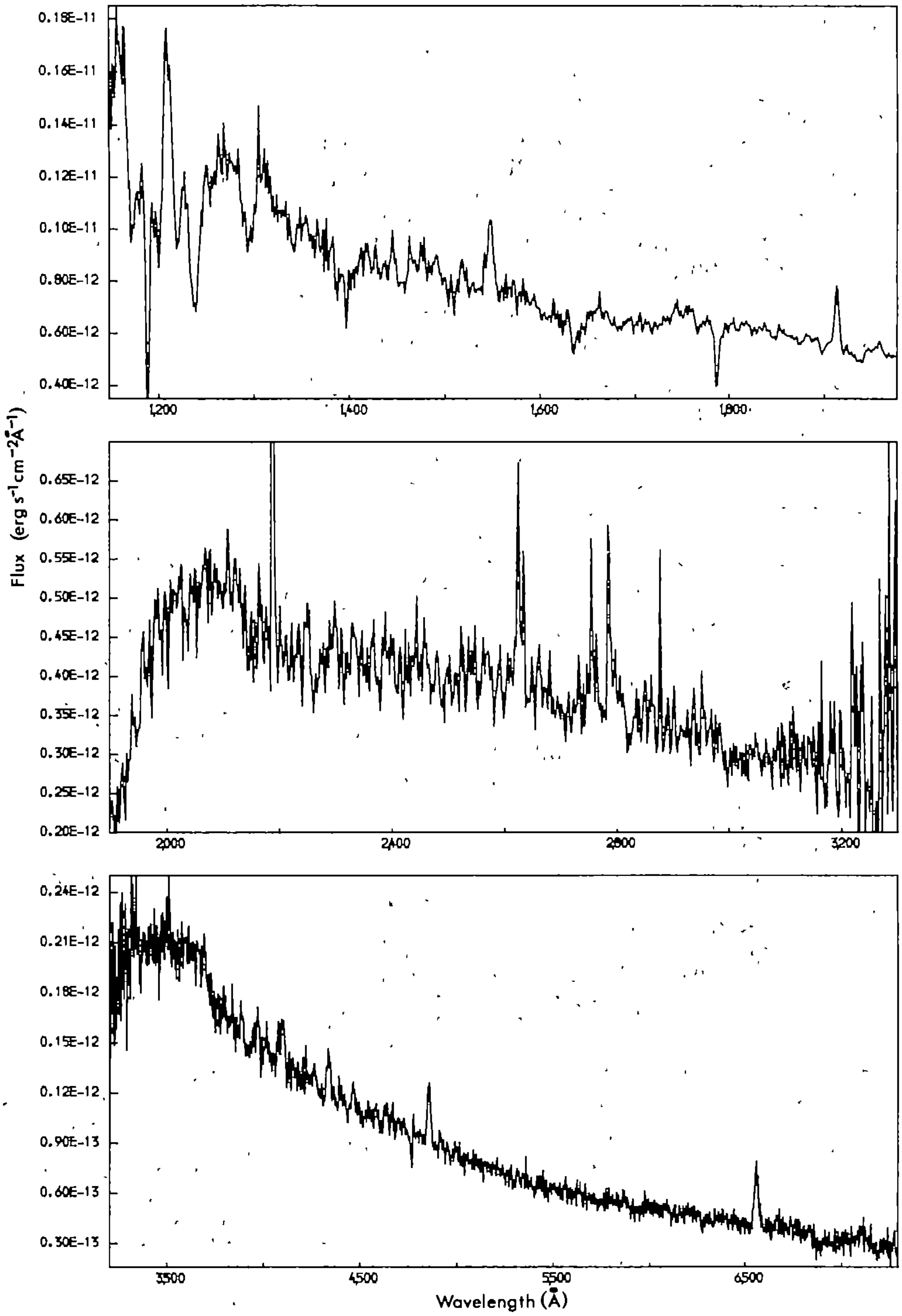

Fig. 1 The short and long wavelength IUE spectra together with the optical spectrum of TT Ari.

The FES curve apparently shows two maxima within the photometric period of 3 h 11 min making it not immediately recognizable as a typical light curve for TT Ari. This is not surprising remembering that there are only nine points (each of ~1 min integration) and that TT Ari shows considerable flickering. The larger of these two maxima can, however, be identified as the usual maximum since its epoch fits with the photometric period to some optical observations carried out in November 1981. A secondary maximum is also apparent in some of the published light curves. Ideally observations should have continued for two or more periods but this was not possible since the IUE satellite was beginning to overheat in this pointing direction. Also the 20 min preparation time needed for the IUE cameras makes it impossible to improve the time resolution without resorting to 'trailed' spectra which are difficult to analyse.

The reason for this changing amplitude can be seen from Fig. 3 which shows the five UV wavelengths together with *U*, *B* and *V* points derived from the FES counts and the known colours and changes in colours, taken from ref. 2. It is clear that the spectrum of TT Ari has two components, an unchanging black-body-like component which peaks near 3,000 Å, and a rather flat (in frequency units) component which causes the variability. This flat spectrum must also turn down in the optical region. The change between the two spectra is approximately the same (~20 mJy) at each wavelength, suggesting the source of the flat spectrum is seen at differing aspects.

It is possible allowing for errors that the whole spectrum from 5,500 to 1,265 has a single amplitude of ~1.4 mag. However, the shape of the spectrum lends credence to a change of amplitude with wavelength. It is just conceivable that a disk atmosphere model might explain the whole observed spectral shape from 5,500 to 1,265 Å; this would then all have to vary with the single amplitude. However, the correlation of the UV and X-ray variation makes such a model implausible.

These observations cannot be explained by a hot spot on the edge of an accretion disk. Large variations (amplitude 1.53) are seen at $\lambda = 1,265$ Å; radiation at this wavelength would be expected to arise close in to the white dwarf and not from the edge of an accretion disk. Any modulation by the hot spot is the more difficult when it is remembered that since there are no eclipses the inclination (i) must be <60°. Finally X-ray observations by Jensen *et al.*[9] rule out a hot spot. They find that 0.1–4 keV X rays vary over the orbital period with an amplitude of ~1.5 in a manner similar to the optical variation which they measured simultaneously. They also find X-ray flickering which correlates with the optical flickering, the latter preceding the X-ray flickering by $\gtrsim$40 s. This strange result may be due to a quasi-periodic flickering time of ~40 s found by Mardirossian *et al.*[3]. Clearly, the X-ray variation also rules out a model in which the variations arise from a non-axisymmetric atmosphere to the accretion disk, even if a way could be found of maintaining this asymmetry for many orbital cycles so as to produce the light curves. That optical, UV and X-ray radiations all vary in broadly the same manner cannot be consistent with a hot spot model.

This behaviour is exhibited by the AM Herculis stars, and an AM Her model might therefore seem attractive. However, it too can be ruled out. The constant black-body component of the spectrum of TT Ari is distorted by the Balmer jump in emission. Estimating its temperature, therefore, as ~12,000 K from *UBV* colours rather than from its apparent peak emission at 3,000 Å we can then calculate its area (taking $i = 45°$) as $\sim 10^{20}$ cm^2. There is no room for such a large constant black body in the usual model for an AM Her type system.

The observations do, however, fit a DQ Herculis type model with a slowly (synchronously, or almost so) rotating white dwarf. The constant 'black body' is due to an accretion ring rather than a disk. At the inner edge of this ring the accretion is funnelled down the magnetic field of the white dwarf. The accretion spot at the base of the magnetic accretion columns is thought to have two components (see ref. 10): a hot, optically thin region which produces the hard X rays, and below this a photospheric region radiating most of the accretion luminosity with a black-body-like spectrum ($T \sim 3 \times 10^5$ K) which dominates the EUV and the soft X-ray region. If the magnetic field of the white dwarf is offset from the rotation axis the changing aspect of the accretion spot provides a natural explanation for the optical and UV variation. We also note that the absorption lines such as N V, Si V and He II may be formed in the hot photospheric region. More plausibly, any wind flowing from the system may cause these absorption lines.

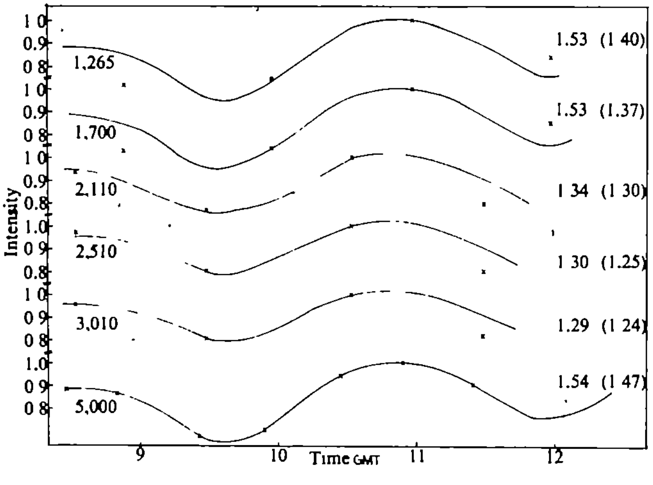

Fig. 2 The 1,265, 1,700, 2,110, 2,510 and 3,010 and 5,000 Å light curves of TT Ari. Fluxes are normalized such that maximum light is unity. The numbers on the right give the amplitudes (maximum/minimum) of the curves and in parentheses the amplitudes derived from the observations alone.

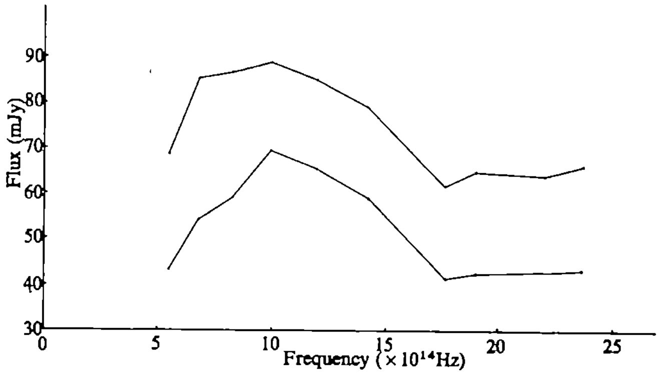

Fig. 3 The maximum and minimum light spectra of TT Ari. 1,360 Å and 1,580 Å have been added.

The sinusoidal modulation of the hard X rays is more difficult to understand as they must come from an optically thin region and there is no evidence for eclipses (we always see just one magnetic pole) or absorption events (K. O. Mason, personal communication). Such sinusoidal modulations are seen in the hard X-ray light curves of some AM Her systems (such as EF Eri = 2A0311 − 227 (ref. 11), where there is also an absorption event, presumably due to the accretion stream crossing the line-of-sight). It is hard to believe that these modulations are caused by electron scattering by material outside the X-ray source: apart from the difficulty of holding the scattering material in place so as to produce regular light variations, any plausible arrangement will also give photoelectric absorption events, contrary to observation. This cannot be overcome by photoionization, since to remove the *K*-shell electrons from the absorbing elements requires[12] $L_x \gg 10^3 N_e r^2$, where L_x is the X-ray luminosity, r the distance from the X-ray source and N_e the average electron density in the scattering material. As the scattering optical depth $\tau_{es} \lesssim N_e r \sigma_T$ must be $\gtrsim 1$ and $r \gtrsim 10^{10}$ cm in any plausible configuration, we require $L_x \gg 10^3 r \tau_{es}/\sigma_T \gg 10^{37}$ erg s^{-1} to get scattering without absorption, considerably in excess of the total luminosity of TT Ari. This would also be a serious problem for a model invoking hard X-ray production in the boundary layer of an accretion disk extending down to the white dwarf surface. A possible explanation for the hard X-ray variation in an accretion spot model comes from the fact that half of the emitted X rays hit the stellar surface: of these some fraction a_x are scattered outwards again. This scattered (albedo) component will be sinusoidally modulated by an aspect effect just like the soft X rays and EUV. Clearly this idea will work best if a purely scattering albedo ($a_x \sim 0.7$) (ref. 13) as opposed to a scattering plus photoelectric absorption albedo ($a_x \sim 0.3$) (ref. 13) is appropriate.

A second difficulty for our model is the shape of the UV continuum, which is much flatter than the Rayleigh–Jeans continuum ($F_\nu \propto \nu^2$) expected from the accretion spot. We note that in AM Her the UV continuum is $F_\nu \sim \nu^{-1}$, although the eclipsed component does have $F_\nu \propto \nu^2$ (ref. 14). X-ray reprocessing is suspected in AM Her, and may be operating here.

Some cyclotron radiation may be expected from material in the accretion columns. However, because the magnetic field must be weaker than in AM Her this is only likely to contribute in the IR. Furthermore, any such radiation will be heavily diluted by the IR tails of the accretion spot, accretion ring and the red star. Neverthless, it would be interesting to look for such weak polarization in the IR. TT Ari is known to be unpolarized in the optical region[15,16].

The variation of the C IV, Mg II emission lines is puzzling as these must, in any model, come from an optically thin region. Radial velocity studies are needed to establish the likely sites of these emission regions. Modulation could arise if they are near the heated inner face of the secondary or the base of the accretion columns. We note that large emission line variations are seen in the magnetic rotator EF Eri[17].

The period of rotation of the white dwarf must be the photometric period, 0.1329 days as given by Smak and Stepien[5]. The binary period is, of course, given by the spectroscopic period of 0.13755 days: it would seem therefore that TT Ari has only recently broken away from the synchronism of an AM Her type system.

One might expect to see a reflection effect, due to the reprocessed UV and X rays, off the secondary. This will be best seen when the secondary is viewed over the white dwarf and the magnetic pole of the white dwarf points towards the secondary. This situation will recur every 3.93 days. Observations of such an effect would test our model and confirm the spectroscopic and photometric periods. We note, however, that the predicted ~4-day period is very sensitive to errors in the photometric and spectroscopic periods. In particular if these periods are closer than current values suggest the 'beat' period may become unobservably long.

We also note that the quasi-periodic variations on time scales of about 14 min (ref. 4) may be related to the free fall time down the accretion columns. This would imply an inner orbital period of the accretion ring of ~56 min. This would be variable as the Alfvén radius varies with the kinetic energy density of the ring which in turn depends on the mass transfer rate.

Clearly, our model describing TT Ari cannot be regarded as certain. Many of the problems, however, are caused by a lack of detailed theoretical understanding of the processes associated with accreting magnetized white dwarfs, for example the X-ray variation problem in a non-eclipsing system such as EF Eri. Equally clear is that a classical hot spot cannot explain the UV and X-ray variation. The axisymmetry of models involving an accretion disk with X rays generated at the white dwarf boundary layer makes it very difficult to modulate the short wavelength UV and X rays, because absorption events are not seen and scattering can be ruled out.

Although we have described TT Ari as a DQ Her type system it is perhaps truer to say it is intermediate between the DQ Her and AM Her types. Indeed, one can see a sequence of objects DQ Her (71), H22521035 (805), TT Ari (11,474) and AM Her (synchronized) where the numbers in parentheses represent the spin period of the white dwarf in seconds. Of course, if the spectroscopic period is in error and does equal the photometric period then TT Ari would be an unusual type of AM Her system in which the magnetic field was weak enough to allow the formation of an accretion ring.

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Limits on a radio shell around the Crab Nebula

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It has been suggested[1] that the supernova of AD1054, which produced the Crab Nebula, was of Type II and resulted from the explosion of a massive (10–15 $M_\odot$) star. In this picture, the well known optical filaments represent material which originated predominantly in the core of the star and has been accelerated outwards by the pulsar activity. This nebula is supposed to be surrounded by a faint, massive (~10 $M_\odot$), high velocity (≥5,000 km s^{-1}) shell[2] or halo, which was the envelope of the presupernova star. Such an outer halo may have been recently detected at optical wavelengths[3]. We report here a high sensitivity search at radio wavelengths for the hypothetical outer shell. No shell has been detected and the upper limits indicate that any such shell is fainter than expected from the surface brightness—linear diameter (Σ–D) relation for shell type supernova remnants. Alternative explanations for the optical halo[3] are mentioned.

Observations were made in 1975 and 1976 with the Westerbork Synthesis Radio Telescope[4,5] which then consisted of 12 equatorially mounted paraboloidal antennas distributed over 1.5 km along an east–west line. In searching for a hypothetical radio shell around the Crab Nebula, it is desirable to observe at a low frequency, as the spectrum of the shell is expected to be steeper than that of the pulsar driven nebula, which has $\alpha = 0.26$ ($S \propto \nu^{-\alpha}$) (ref. 6). The frequency used, 610 MHz, provided excellent 'dynamic range' and sensitivity to low brightness features, as well as adequate resolution (55 × 151 arc s, $\alpha \times \delta$, FWHM). As the observations were primarily intended for mapping the weak linear and circular polarization of the nebula, a special observing technique was used[7]. The procedure involved two 12-h observations of the Crab Nebula, one with the two perpendicular linearly polarized dipoles in each telescope at position angles (PA) 0° and 90° and the second with them at PA 45° and 135°. Because of the great brightness of the central nebula, it was necessary to attenuate the signals in the parallel channels on the shorter baselines, to prevent saturation in the correlators. Unnecessary loss of signal-to-noise was, however, avoided by performing greater attenuation on the shorter baselines, in effect 'matching' the sensitivity of the instrument to the visibility curve of the radio source. Each of these 12-h observations was sandwiched between two observations, with the same dipole positions, of the calibration source 3C147 to establish accurately the instrumental polarization and by two observations of the same calibrator with the dipoles in their normal positions[8], to derive the instrumental amplitude and phase corrections. This entire sequence was performed once in March 1975 and again in January 1976, resulting in four independent full (12 h) syntheses of the total intensity distribution. These four maps are in excellent agreement and we use mainly the results of the first series of observations, as the 'dynamic range' is better. Results of the polarization mapping will be presented elsewhere.

At the present resolution the details seen in the highest resolution centimetre wavelength maps[9,10] are smeared out and the cleaned[11] map of the nebula (Fig. 1) shows a smooth, elongated brightness distribution. Averaging the four individual observations, the total flux density is $S_{610\,\mathrm{MHz}} = 1{,}085 \pm 30$ Jy,

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in good agreement with earlier measurements[6]. The observed major × minor axes of the nebula (with no correction for beam smoothing) are 4.4×3.1 arc min in PA = 152° at 50% of the peak intensity (FWHM) and 10.4×7.6 arc min in PA ≃ 150° at 0.5% of the peak intensity. Assuming a gaussian brightness distribution, the approximate deconvolved[12] sizes are 3.8×2.6 arc min in PA 134° to FWHM, and 8.9×5.6 arc min PA 124° at 0.5%. This deconvolved FWHM is similar to other measurements at centimetre wavelengths[9]. Although the deconvolution is obviously not precise, the size at the 0.5% level may be significantly larger than the total size of 7×5 arc min found at 5 GHz (ref. 9), presumably because of the higher 'dynamic range' of the present data. It is also slightly larger than the optical dimensions of 7×4.8 arc min given by van den Bergh[13]. About 5 arc min to the north of the pulsar, a radio protrusion is seen in the second and third contours. This protrusion lies close to, and slightly to the north of, the optical 'jet-like' feature[13,14]. However, the low resolution in the declination direction renders the position of the radio feature uncertain and higher resolution data are needed to check for a possible association. The extensions to the north seen in the first contour are probably not real.

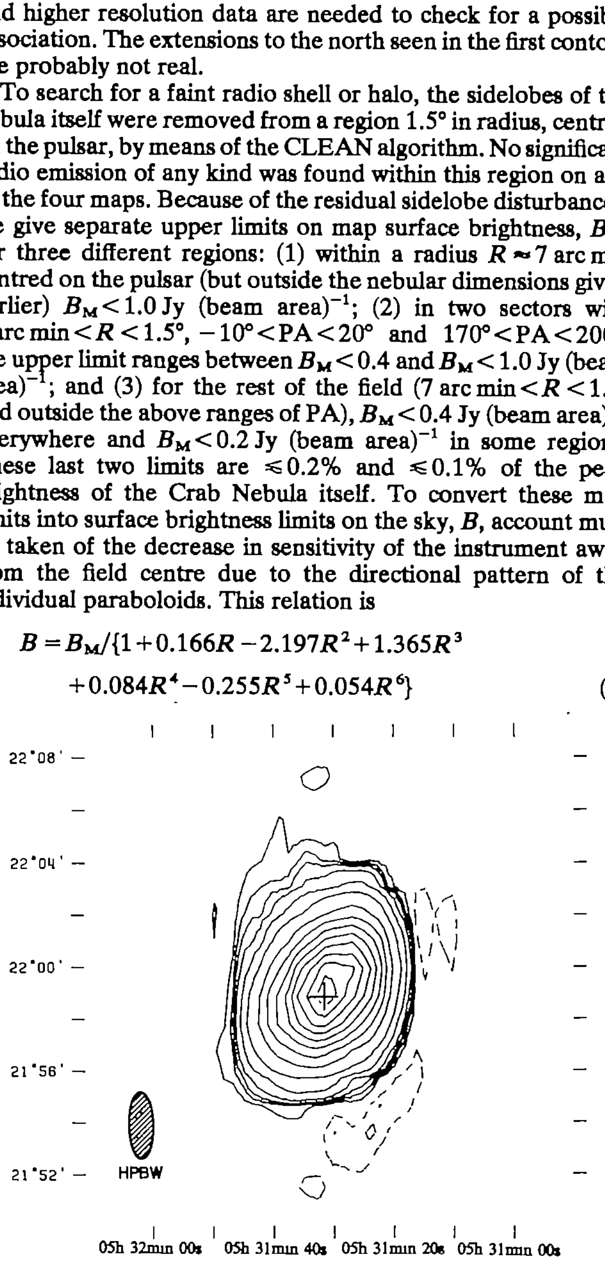

Fig. 1 Contour map of the total intensity distribution in the Crab Nebula at 610 MHz. The beam (55×151 arc s, $\alpha \times \delta$) is shown at the bottom left and the cross marks the pulsar. Contours are plotted at: −2, −1, 1, 2, 3, 5, 10, 20, 40, 60, 80, 100, 120, 140, 160, 180 Jy (beam area)$^{-1}$. Negative contours are dotted lines, positive contours are solid lines.

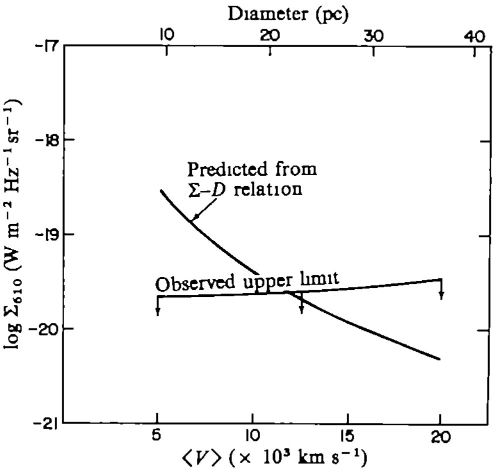

Fig. 2 Plot of surface brightness at 610 MHz (Σ_{610}) against present linear diameter or mean expansion velocity for a hypothetical radio shell around the Crab Nebula at a distance of 2 kpc. The expected mean surface brightness from the Σ–D relation[15] ($|z| = 200$ pc) and the observed upper limit are shown.

To search for a faint radio shell or halo, the sidelobes of the nebula itself were removed from a region 1.5° in radius, centred on the pulsar, by means of the CLEAN algorithm. No significant radio emission of any kind was found within this region on any of the four maps. Because of the residual sidelobe disturbances, we give separate upper limits on map surface brightness, B_M, for three different regions: (1) within a radius $R \approx 7$ arc min centred on the pulsar (but outside the nebular dimensions given earlier) $B_M < 1.0$ Jy (beam area)$^{-1}$; (2) in two sectors with 7 arc min $< R < 1.5°$, $-10° < \mathrm{PA} < 20°$ and $170° < \mathrm{PA} < 200°$, the upper limit ranges between $B_M < 0.4$ and $B_M < 1.0$ Jy (beam area)$^{-1}$; and (3) for the rest of the field (7 arc min $< R < 1.5°$ and outside the above ranges of PA), $B_M < 0.4$ Jy (beam area)$^{-1}$ everywhere and $B_M < 0.2$ Jy (beam area)$^{-1}$ in some regions. These last two limits are $\leq 0.2\%$ and $\leq 0.1\%$ of the peak brightness of the Crab Nebula itself. To convert these map limits into surface brightness limits on the sky, B, account must be taken of the decrease in sensitivity of the instrument away from the field centre due to the directional pattern of the individual paraboloids. This relation is

$$B = B_M/\{1 + 0.166R - 2.197R^2 + 1.365R^3 + 0.084R^4 - 0.255R^5 + 0.054R^6\} \quad (1)$$

where R is the distance from the pulsar ($\alpha(1950.0) =$ 05 h 31 min 31.46 s, $\delta(1950.0) = +21°58'54.8''$) in degrees. Thus the upper limit on B is just twice the upper limit on B_M at 41 arc min from the field centre and is negligibly (7%) larger than B_M at 14 arc min (the maximum radius of the optical halo[3]). These upper limits on B may, in turn, be converted into limits on conventional surface brightness by means of

$$\Sigma_{610}(\mathrm{W\,m^{-2}Hz^{-1}sr^{-1}}) = 5.5 \times 10^{-20} B\,(\mathrm{Jy(beam\ area)^{-1}}) \quad (2)$$

Thus for the typical upper limit of $B < 0.4$ Jy (beam area)$^{-1}$, equation (2) implies $\Sigma_{610} \lesssim 2 \times 10^{-20}$ W m^{-2} Hz^{-1} sr^{-1}.

We may compare our limits with the surface brightness–linear diameter (Σ–D) relation for shell-type supernova remnants. While this relationship is empirical and of weak predictive power, it remains the major yardstick for studying the radio evolution of supernova remnants. Using the form determined by Caswell and Lerche[15] and converting from a surface brightness at 408 MHz to one at 610 MHz, assuming a mean spectral index $\alpha = 0.45$ (ref. 16), gives the relation

$$\Sigma_{610}(\mathrm{W\,m^{-2}\,Hz^{-1}\,sr^{-1}}) = 0.8 \times 10^{-15} D_{pc}^{-3} \exp(-|z_{pc}|/175) \quad (3)$$

where Σ_{610} is the mean surface brightness of the remnant and D_{pc} and z_{pc} are its linear diameter and height above the galactic plane. A shell of radius 14' around the Crab Nebula at a distance of 2 kpc ($D = 16.3$ pc) and a height above the galactic plane of $|z| = 200$ pc (ref. 17) would, therefore, be expected to have a mean surface brightness of $\sim 6 \times 10^{-20}$ W m^{-2} Hz^{-1} sr^{-1} and at least a factor of two higher rim brightness for a typical shell. As this value exceeds our radio brightness limit by $>3\sigma$, it is clear that if the optical halo detected by Murdin and Clark[3] represents the remnant of the supernova of AD1054, it is atypically weak in the radio range.

A more general way of considering this problem is shown in Fig. 2. The observed upper limit to the 610 MHz surface brightness over most of the map area is shown and compared with the empirical mean surface brightness for shell type remnants from equation (3). As any associated shell is presumed to have an age identical to that of the Crab Nebula itself, an average expansion velocity for each shell size can also be calculated for the abscissa in Fig. 2.

As may be seen, the observed limit lies below the mean expected value for all expansion velocities below $\sim 12{,}000$ km s^{-1}. In fact, because most remnants show appreciable limb brightening, the actual surface brightness in the shell itself would be larger than the mean value computed from the Σ–D relation. Thus the radio observations conflict with the predictions for essentially all plausible expansion velocities.

Therefore, we conclude that any hypothetical shell radio source is fainter than expected from the Σ–D relation. We emphasize that a shell like that of SNR1006 (G327.6+14.5) (ref. 18) would have been barely detected by our observations. However, this source is very faint for its diameter, a fact which may be ascribed to its large distance from the galactic plane ($|z| = 340$, 575 or 1,012 pc, depending on whether the distance is derived from ancient historical records[19], from the $\Sigma(D, z)$ relation[15] or from a $\Sigma(D)$ relation[16]), as its properties are not inconsistent with equation (3).

Theoretical treatments of the radio emission from young supernova remnants commonly rely on an interaction between the ejected material and the surrounding interstellar gas to generate the requisite cosmic rays and magnetic fields[20,21]. In Gull's model[20], the radio emission rises to a maximum when the ratio of mass swept up ($= 1.3 < V_{5,000}^3 > n_{0\,1}\, M_\odot$ for the Crab, where $n_{0\,1}$ is the ambient interstellar density in units of $0.1\ \mathrm{cm}^{-3}$) to mass ejected ($\simeq 10\, M_\odot$ in Chevalier's model[1]) is about 0.1. This model suggests, therefore, there should be significant radio emission from the shell at present. Obviously more quantitative models of the evolution of the radio emission from young shell remnants are needed before comparison with our limits becomes meaningful.

Various possible interpretations of the optical halo[3] include: (1) emission from the hypothetical remnant shell; (2) a 'Strömgren sphere' in interstellar gas or in gas lost by the presupernova star, which is ionized by the non-thermal UV synchrotron radiation of the nebula[22]; (3) light scattered off dust grains surrounding the nebula[3]; and (4) leakage of synchrotron emitting electrons from the nebula[23]. The non-spherical shape of the halo[3] may render (2) implausible while any interpretation, such as (3), must be consistent with the apparent absence of excess dust absorption in and surrounding the Crab[24], as well as the halo's emissivity in the band straddling Hα used by Murdin and Clark[3]. The most direct way to prove possibility (1) would be the detection of high velocity gas in the halo through optical spectroscopy; in fact, Henry *et al.*[25] have recently claimed the detection of gas expanding at velocities of 2,300–7,200 km s^{-1}. Further X-ray observations are also desirable to confirm and map a possible soft X-ray 'halo' peripheral to the nebula[26], although this halo is probably the result of scattering of X rays from the central synchrotron source by interstellar dust rather than emission from a hot thermal plasma[27].

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Note added in proof: R. B. C. Henry has informed us that the high-velocity emission lines suspected in ref. 25 are of instrumental origin.

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Simultaneous radio scattering and white light observations of a coronal transient

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Comprehensive observations of coronal transients have been made using the OSO 7 (ref. 1), Skylab[2–4], Solar Maximum Mission[5] and currently Solwind[6,7] coronographs. An important question in regard to understanding these transients and their relationship to solar radio burst observations[8] and *in situ* solar wind measurements[9] is whether the shock front produced by the disturbances corresponds to the white light front. It has recently been demonstrated[10] that spectral broadening measurements of monochromatic spacecraft radio signals represent a useful tool for detecting and studying shocks near the Sun. We now report that a coronal transient observed by the Solwind coronograph off the west limb of the Sun on 24 October 1979 was also seen in the spectral broadening observations of the Helios-2 2.3-GHz radio signal. A comparison of these simultaneous data suggests that in this case the shock front does not coincide with the white-light front but in fact precedes it.

Figure 1 shows four coronal images obtained with the Solwind instrument[6] on 24 October, 1979. The upper images were taken at 0316 and 1258 UT while the lower ones are difference images formed by subtracting the images at 0536 and 0804 UT from that at 0316 UT to show the changes that had taken place in the coronal intensity. The west limb brightening is always present during 0316–0804 UT, but a small loop transient does occur during 0536–0804 UT. The Helios-2 raypath, which is in the ecliptic plane off the west limb of the Sun, moves towards the Sun at the rate of 25 km s^{-1}. 'X' in the 0536–0316 UT difference image marks the location of the Helios-2 raypath at 0536 UT. As can be seen, the Helios-2 raypath is well positioned to observe the transient whose front progressed in an almost westward direction.

Although the front of the moving coronal material is not precisely defined, its speed can be estimated and appears to be very slow. The heliocentric distances of the coronal front at 0536 and 0804 UT are $4.5 \pm 0.5 R_\odot$ and $6.0 \pm 1.0 R_\odot$ respectively, yielding an outward speed (in the plane of the sky) of ~ 120 km s^{-1}. However, because of the uncertainties in location, the range of speeds is 0–240 km s^{-1}. Height–time profiles for both the Helios-2 raypath and the white light coronal front measurements are summarized in Fig. 2.

Figure 3 shows the time history of the spectral broadening bandwidth BW of the Helios-2 2.3-GHz signal on 24 October 1979 during 0200–1400 UT. Values of BW, the full width between frequencies enclosing half the area underneath the RF spectrum[10,11], were computed using an integration time of 2 min. The increase in BW depicted in Fig. 3 exceeds that which would be expected due to the systematic increase in background corona towards the Sun[11]. The BW profile in Fig. 3 is similar in shape to that for a shock wave observed by Voyager 1 on 18 August 1979 (Fig. 2*a* in ref. 10), and although we cannot be certain, the similarity in shape indicates that the event in Fig. 3 is also probably a shock wave. Of course, it is clear from the differences in BW variation and time scale that the Helios-observed shock is substantially less intense and slower than the

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Voyager-observed shock. The pre-shock bandwidth of 5 Hz at 2.3 GHz is in agreement with the measurements of the background solar corona near $5R_\odot$ (ref. 11).

Bandwidth increases are caused by enhancements in turbulence (electron density fluctuations) or acceleration of the solar wind[11] and can be separated by analysing the amplitude scintillations if they are weak[10]. Unfortunately, the Helios measurements took place near $5R_\odot$, where the 2.3-GHz amplitude scintillations are saturated and strong[12] so that detailed profiles of the wind speed and turbulence cannot readily be obtained as was done in the case of the Voyager measurements[10]. The first region of enhanced bandwidth in Fig. 3 (0500–0700 UT) corresponds to the shock front and the compressed turbulent region behind it, while the second broad but lower enhanced region (0700–1400 UT) following the first is probably the turbulent wake.

Based on the coronal transient speed of 120 km s^{-1} determined from the Solwind pictures, the white light coronal front would have crossed the Helios-2 raypath at $5.2R_\odot$ around 0700 UT, which is 2 h after the shock arrival time of 0500 UT (see Fig. 3). Alternatively, the 0536–0316 UT difference image in Fig. 1 shows that the white light coronal transient had not reached the Helios raypath at 0536 UT whereas the shock had already been detected 36 min earlier in the Helios radio tracking data. The cross-hatched region in Fig. 2 corresponds to the times at which the white-light front would have crossed the Helios raypath when the range of heliocentric distances at 0536 and 0804 UT is considered. As can be seen, the delay in white light coronal front arrival is at least 1 h for all possibilities. This result is consistent with the fact that path-integrated electron density as determined from radio measurements of phase in the case of the 18 August shock peaks well behind the shock front[10].

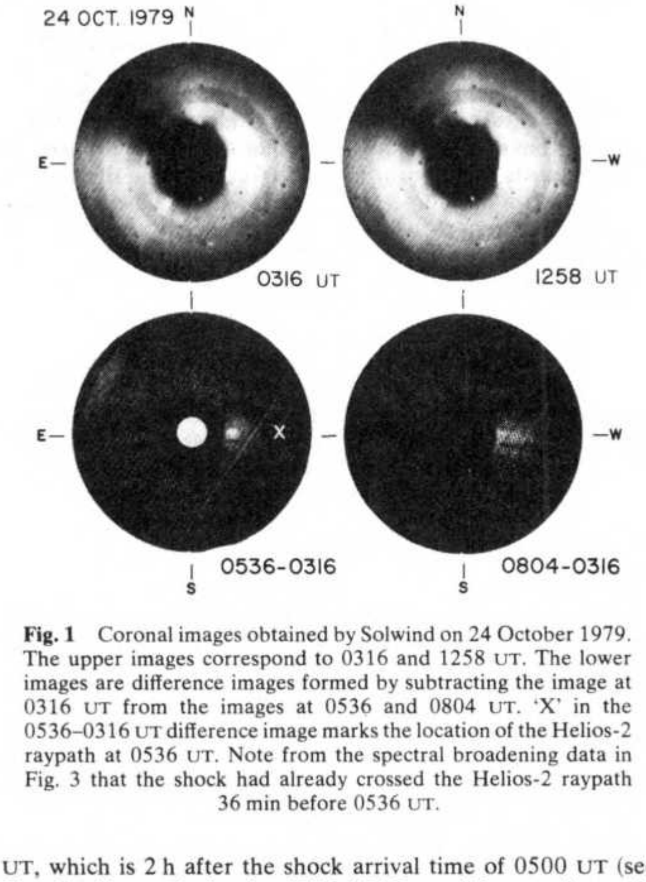

Fig. 1 Coronal images obtained by Solwind on 24 October 1979. The upper images correspond to 0316 and 1258 UT. The lower images are difference images formed by subtracting the image at 0316 UT from the images at 0536 and 0804 UT. 'X' in the 0536–0316 UT difference image marks the location of the Helios-2 raypath at 0536 UT. Note from the spectral broadening data in Fig. 3 that the shock had already crossed the Helios-2 raypath 36 min before 0536 UT.

Let us estimate the shock speed. Assuming that the white light coronal transient has a constant speed of 120 km s^{-1}, the start time at the Sun's surface would have been 2358 UT on 23 October. As discussed earlier, the shock crossed the Helios raypath at about 0500 UT and according to Fig. 2 the heliocentric distance was $5.5R_\odot$. If we assume that the shock originated from the Sun's surface at the same time as the white-light front and travelled at a constant speed to $5.5R_\odot$, we obtain a shock speed of 170 km s^{-1}. The ambient solar wind speed is about 80 km s^{-1} (ref. 12), so the Helios-observed shock must be rather weak. This result is consistent with the fact that the bandwidth increase is substantially smaller for the Helios than for the Voyager-observed shock. It is interesting that the shock speed calculated on the basis of transit time for the Voyager shock was 2,300 km s^{-1} (ref. 13), which is a factor of 13.5 higher than that of the Helios shock. This is approximately the same factor relating the time scales of the bandwidth time histories of the Voyager and Helios shocks. The consistency of the Helios and Voyager spectral broadening observations in this regard reinforces the notion that the event observed by Helios is indeed a shock.

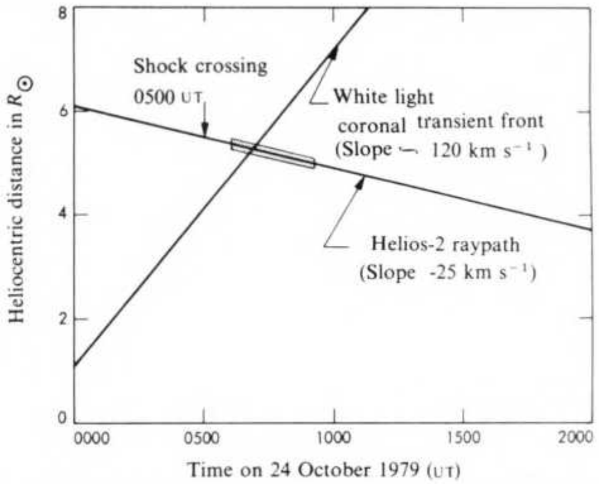

Fig. 2 Height–time profiles for the Helios-2 raypath and Solwind white light coronal front measurements. The cross-hatched region indicates the possible range of intersections between the Helios-2 raypath and the white light transient front allowing for the 120 ± 120 km s^{-1} uncertainty in the measured transient speed.

Because of the paucity of radio scattering measurements using spacecraft radio signals, there have been few opportunities to compare simultaneous radio scattering and white light measurements of coronal transients. The results presented here are based on only one relatively weak coronal transient but clearly show that the shock front is ahead of the white light front. Additional observations of larger events, particularly when solar radio noise burst and *in situ* spacecraft observations are conducted at the same time, will contribute significantly to our understanding of coronal transients and their evolution as they propagate away from the Sun.

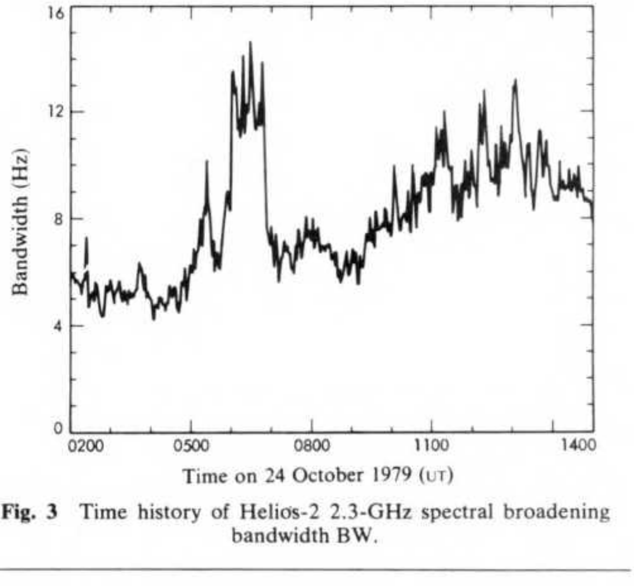

Fig. 3 Time history of Helios-2 2.3-GHz spectral broadening bandwidth BW.

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Aquatic chemistry of plutonium in seasonally anoxic lake waters

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We present here data on the distribution of the fallout radionuclides ^{55}Fe, ^{239,240}Pu, ^{137}Cs and ^{90}Sr and the natural radionuclides ^{238}U and ^{234}U in a seasonally anoxic lake in an effort to determine their biogeochemical behaviour. Results from Gull Pond, Massachusetts show that the oxygen-depleted and iron- and manganese-rich bottom waters contain five times more dissolved ^{239,240}Pu than oxygenated surface waters. This feature, also observed in anoxic hypolimnia of other lakes, indicates that the concentration of plutonium is significantly influenced by anoxicity and redox cycles. Such observations are important in understanding the fate of artificial radionuclides which are released[1–4] to the aquatic environment through global fallout, planned disposal and localized accidents.

The oxidation–reduction chemistries of iron and manganese are important in aquatic systems because they can directly or indirectly control the concentration, distribution and speciation of inorganic and organic constituents[5–7]. In many temperate lakes the development of thermal stratification in summer causes the hypolimnion to become depleted or devoid of dissolved oxygen[5,8–14]. In such conditions, reductive dissolution of sedimentary Fe(III) and Mn(IV) oxides occurs, releasing dissolved Fe(II) and Mn(II) to the overlying water[5,8–14]. Anoxic hypolimnia can have large concentrations of dissolved Fe(II) and Mn(II). Ferrous iron is then rapidly oxidized in the oxygenated part of the water column to form suspended particles of Fe(III) oxides[5–15].

Many natural radionuclides (such as uranium, thorium, radium, ^{210}Pb, ^{210}Po) and artificial radionuclides (such as plutonium, americium) are known to be strongly adsorbed onto the oxyhydroxides of iron and manganese[16,17]. Therefore, the reductive dissolution and oxidative precipitation of iron and manganese oxides in seasonally anoxic lakes may result in changes in the aqueous concentration of several radionuclides. For plutonium and uranium with their multiple oxidation states[18–25], variations in redox conditions may lead to changes in speciation and hence concentrations in lake waters.

Gull Pond, a small (0.5 km^2) kettle hole lake in Wellfleet, Cape Cod, Massachusetts, develops an anoxic hypolimnion during the late summer (Fig. 1). On 19 August 1981, we collected a vertical profile of water from the deepest basinal part of the lake (22 m depth). Large volume water samples for radiochemical analyses were obtained by pumping water from depth up a plastic hose, through an AMC Cuno Micro-Wynd II filter cartridge with a nominal 1 μm pore size and into 60-1 LPE Deldrums. Water samples were filtered without contact with the atmosphere. Such handling is necessary with anoxic waters to prevent the rapid oxidation of dissolved Fe(II) iron to particulate Fe(III) oxide particles. If this reaction did occur before filtration, then the Fe(III) oxides thus formed would probably scavenge dissolved nuclides resulting in the measured dissolved and particulate nuclide concentrations being too low and too high respectively.

Dissolved (<1 μm) radionuclides, iron and manganese were measured on the 2, 15 and 20 m samples. Using Niskin bottles, a series of smaller samples were collected for analysis of dissolved O_2 and total iron and manganese. Oxygen was measured by the Winkler titration and iron and manganese by flame atomic absorption spectrometry. The radiochemical analyses were performed according to published methods—^{239,240}Pu (ref. 45), U (ref. 46), ^{55}Fe (refs 26, 27), and ^{137}Cs (ref. 28) and ^{90}Sr (ref. 28).

The seasonal anoxicity of the hypolimnion in Gull Pond has been described elsewhere[29]. During our August sampling (Fig. 1) there was a large decrease with depth in the concentration of dissolved oxygen in the thermocline (9–16 m); and an anoxic hypolimnion existed between 16 m and the bottom (22 m). The oxygen decrease was accompanied by large increases with depth in the concentrations of total iron and manganese. These two elements were predominantly in the dissolved form (<1 μm; Table 1). The iron and manganese gradients are typical of those observed in other seasonally anoxic lakes[5,10–12,14].

The concentration of dissolved ^{239,240}Pu has a 4.6-fold increase between 2 and 20 m with the larger gradient occuring in the anoxic hypolimnion between 15 and 20 m. The gradient for plutonium is much greater than those of dissolved ^{137}Cs, ^{90}Sr and U (Fig. 1). The plutonium profile is more similar to that of ^{55}Fe which has a steep gradient and follows that of stable iron. Iron-55 (half life of 2.7 yr) was introduced with plutonium in the global fallout from atmospheric nuclear explosions testing[30]. The data from Gull Pond show that dissolved ^{239,240}Pu concentrations are higher in the anoxic hypolimnion. The relative amounts of plutonium in the dissolved and particulate phases have not been measured. Release from the sediments to the water column in anoxic conditions is suggested by the similarities in the plutonium and ^{55}Fe gradients.

The only published studies of plutonium distributions in anoxic lakes are those of Experimental Lake 241, a meromictic Canadian lake with permanently anoxic bottom water[31–33] and Par Pond (S. C.), a lake with a seasonally anoxic hypolimnion[34]. In ELA-241 the dissolved ^{239,240}Pu concentration increases from 6 to 10 fCi l^{-1} between 0 and 9 m, then increases to 48 fCi l^{-1} at 11 m (Fig. 2). The bottom water has high (but unspecified) concentrations of dissolved iron and manganese and displays a large increase in conductivity[31–33]. Wahlgren *et al.*[32,33] have determined that ~80% of the dissolved ^{239,240}Pu in ELA-241 is in the reduced (III+IV) state.

Four vertical profiles (Fig. 2) of ^{239,240}Pu in Par Pond reveal variations which may be linked to redox cycling. The profile in February 1976 shows no vertical gradient when the lake is well mixed. Profiles in July 1975 and August 1976 show the deepest samples up to 20 times more concentrated in plutonium than those in the overlying water. Albert and Orlandini[34] reject their

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Table 1 Composition of water column profiles in Gull Pond, 19 August 1981

Non-radioactive constituents

Depth (m)	O_2 (ml l^{-1})	Temperature (°C)	Total Fe (μmol l^{-1})	Dissolved* Fe (μmol l^{-1})	Total Mn (μmol l^{-1})	Dissolved* Mn (μmol l^{-1})
0	6.3	23.7	<0.1			
2	—	—	—	<0.2	—	1.1
3	6.6	23.6	<0.1		0.7	
6	6.1	23.4	<0.1		0.4	
9	6.3	23.0	<0.1		0.7	
10.7	7.2	18.8	<0.1		0.4	
12	5.6	14.2	0.3		1.3	
15	—	—	—	54	—	7.3
15.5	0.28	10.5	52		6.9	
19	0	11.0	125		7.3	
20	—	—	—	112	—	6.0

Radioactive constituents—dissolved*

Depth (m)	$^{239,240}Pu$ (fCi l^{-1})	^{137}Cs (fCi l^{-1})	^{90}Sr (fCi l^{-1})	^{55}Fe‡ (d.p.m. per 100 kg)	^{238}U (d.p.m. per 100 kg)	^{234}U (d.p.m. per 100 kg)
2	0.17±0.02†	323±2	450±5	0.7±0.8	0.196±0.022	0.205±0.021
15	0.26±0.05	387±2	513±9	29.3±1.1	—	—
20	0.76±0.05	451±4	573±9	63.0±1.2	0.283±0.025	0.329±0.027

Activity ratios of dissolved radionuclides

Depth (m)	$^{55}Fe/^{239,240}Pu$	^{55}Fe(d.p.m.)/Fe(g)	$^{137}Cs/^{90}Sr$	$^{234}U/^{238}U$
2	19±22	—	0.71±0.01	1.05±0.13
15	514±101	97±4	0.75±0.01	—
20	371±33	100±2	0.79±0.01	1.16±0.14

* Dissolved refers to water passing through a Micro-Wynd Cartridge with a 1 μm nominal pore size.

† Precisions are 1σ uncertainties from counting statistics.

‡ ^{55}Fe data decay corrected to date of collection (19 August 1981).

high bottom water concentrations of dissolved plutonium in July 1975 as an artefact, do not discuss the high bottom value in total plutonium in the August 1976 profile, and conclude that plutonium is not involved in the redox cycles of iron and manganese.

The results of both studies are ambiguous in that they are not accompanied by basic data indicative of the redox reactions occurring in the lakes at the time of sampling (for example, dissolved O_2, particulate and dissolved iron and manganese). Moreover, it is not clear what precautions were taken to prevent oxidation of dissolved ferrous iron before filtration.

We conclude that the Gull Pond data presented here, supported by the ELA-241 and Par Pond results, indicate that significantly higher dissolved plutonium concentrations are found in anoxic lake waters than in overlying oxic waters. We suggest that the plutonium gradients result from the release of plutonium to water column from the sediments during periods of anoxicity. The exact geochemical processes remain to be established; the possibilities include: (1) plutonium, adsorbed onto sedimentary oxides of iron and/or manganese, is released to the water column as the oxides are reductively solubilized in anoxic conditions[17,34]; and (2) redox transformations lead to a more soluble oxidation state of plutonium possibly through the formation of organic-Pu(IV) complexes (D. M. Nelson, personal communication); chemical modelling[24,25] suggests that in anoxic conditions in lakes the concentration of plutonium will increase due to the higher solubility of Pu(III) in equilibrium with $Pu_2(CO_3)_3$.

The concentrations of ^{137}Cs, ^{90}Sr, ^{234}U and ^{238}U increase only slightly (1.3–1.6-fold) with depth from the surface to the hypolimnetic sample. In comparison with the Great Lakes[35–38], the Gull Pond waters have similar concentrations of dissolved ^{90}Sr but are greatly elevated in ^{137}Cs. The ratios of $^{137}Cs/^{90}Sr$ in Gull Pond range from 0.71 to 0.79. This ratio is approxi-

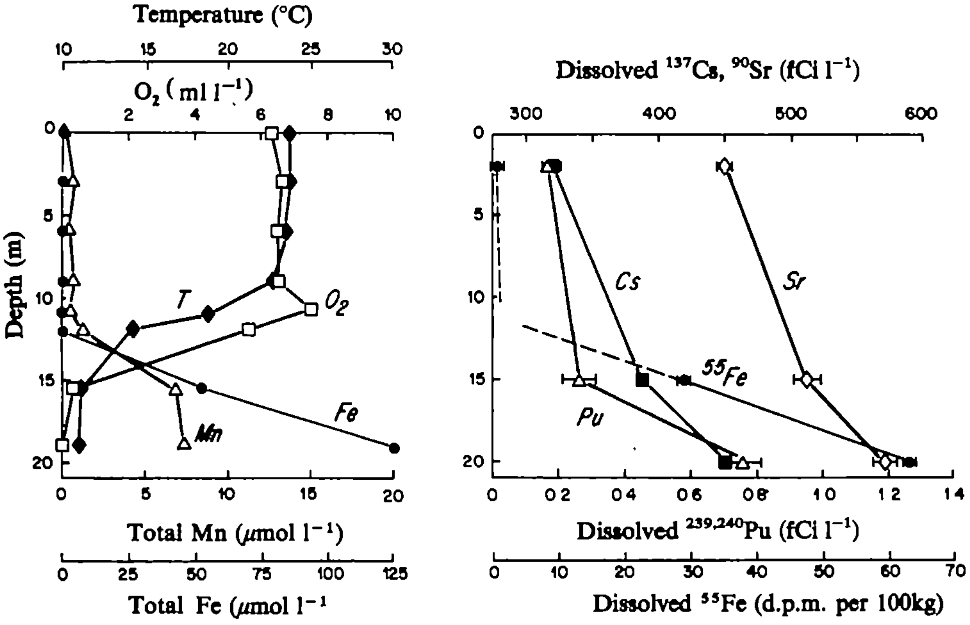

Fig. 1 Chemical composition of the water column in Gull Pond on 19 August 1981. The dashed lines for ^{55}Fe, which intersect at 12 m, are based on the more complete profile of stable Fe. Dissolved refers to constituents passing through a Micro-Wynd Cartridge filter with a nominal pore size retention of 1 μm. Water depth at the sampling location was 22 m.

mately half that of global fallout[39] (1.6 ± 0.2) and 5–20 times greater than ratios measured in the Great Lakes[36,37]). The low clay and high sand content of the soils and lake sediments of Cape Cod may lead to diminished exchange capacities and less uptake of dissolved ^{137}Cs from lake and ground waters.

For Gull Pond the water column (0–20 m) inventories of ^{137}Cs and ^{90}Sr are 8 and 10 mCi km^{-2}. These represent about 7 and 12% of the fallout delivery at this latitude. The water column ^{239,240}Pu inventory is 3.5×10^{-3} mCi km^{-2}, only 0.4% of the fallout delivery. These inventory values are calculated for the deepest part of the lake where our samples were taken and thus are upper limits. They indicate that a major fraction of the fallout nuclides are not in the water column.

The different vertical gradients of ^{137}Cs, ^{90}Sr and U relative to Pu and ^{55}Fe imply that there may be different geochemical and/or hydrological processes operating on these groups of radionuclides. It can be argued that the slightly increasing concentrations in Cs, Sr and U may be due to multiple water sources to the pond, redox controlled release from sediments, or biological removal in the surface water and release at depth. In lakes like Gull Pond, which are predominantly ground water fed, the influence of ground water hydrology on the water column chemistry may be an important unknown[40].

There is some evidence from other anoxic lakes that the Fe/Mn redox cycle may be responsible for increases in the hypolimnetic concentrations of ^{137}Cs (refs 41, 42) and ^{90}Sr (ref. 47). U concentrations are thermodynamically predicted and observed to be very low in anoxic groundwaters[18,19]. However, in anoxic marine sediments, U pore water concentrations are elevated over those of oxygenated seawater[42–44]. The formation of more soluble complexes of U(IV) with organic matter and/or carbonate may be responsible. Because U can exist in several oxidation states and form complexes, it is not unreasonable to expect it to be involved in redox and biogeochemical cycles. This is not usually considered to be the case for Cs and Sr. However, a recent study[5] of a seasonally anoxic lake shows that cations such as K, Mg, Ca and Ba are indirectly involved in the redox cycles. More geochemical and radiochemical data from seasonally anoxic lakes will elucidate the magnitude of fluxes between sediments and overlying water, the extent of post-depositional remobilization and redistribution, and the interaction between suspended particles and the water.

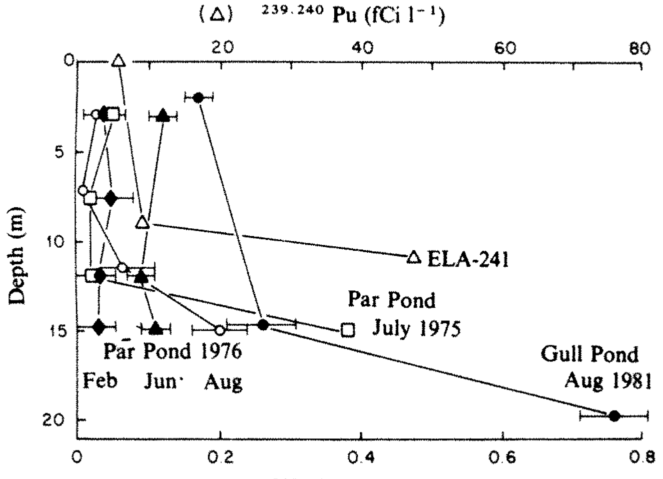

Fig. 2 Profiles of dissolved ^{239,240}Pu in lakes with anoxic hypolimnia. See text for discussion. Water samples from Par Pond (Cold Dam), ELA-241 and Gull Pond were filtered through 0.45, 0.22 and 1.0 μm, nominal sized filters respectively; the only exception is the August 1976 data from Par Pond which are for unfiltered water samples. The maximum water depths are respectively about 16, 12 and 22 m.

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Preferential elution of strong acids from a Norwegian ice cap

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The release of strong acids by melting snows during the early thaw has a serious effect on the ecology of many sensitive areas of the world[1–3]. The run off is a product of both the input (wet and dry deposition) and of processes occurring within the snowpack and the firn during the melt season. Temperate glacial ice is very pure[4], having lost most of its annual input of original solutes during each melt season, and the analyses reported here of the ionic concentrations in the snow, firn and ice of the top 20 m of an ice cap, indicate that the ions are eluted differentially and in a manner which initially accentuates the loss of sulphuric and nitric acid. Almost all the annual deposits of ions on a glacier or ice cap may be lost during a single year.

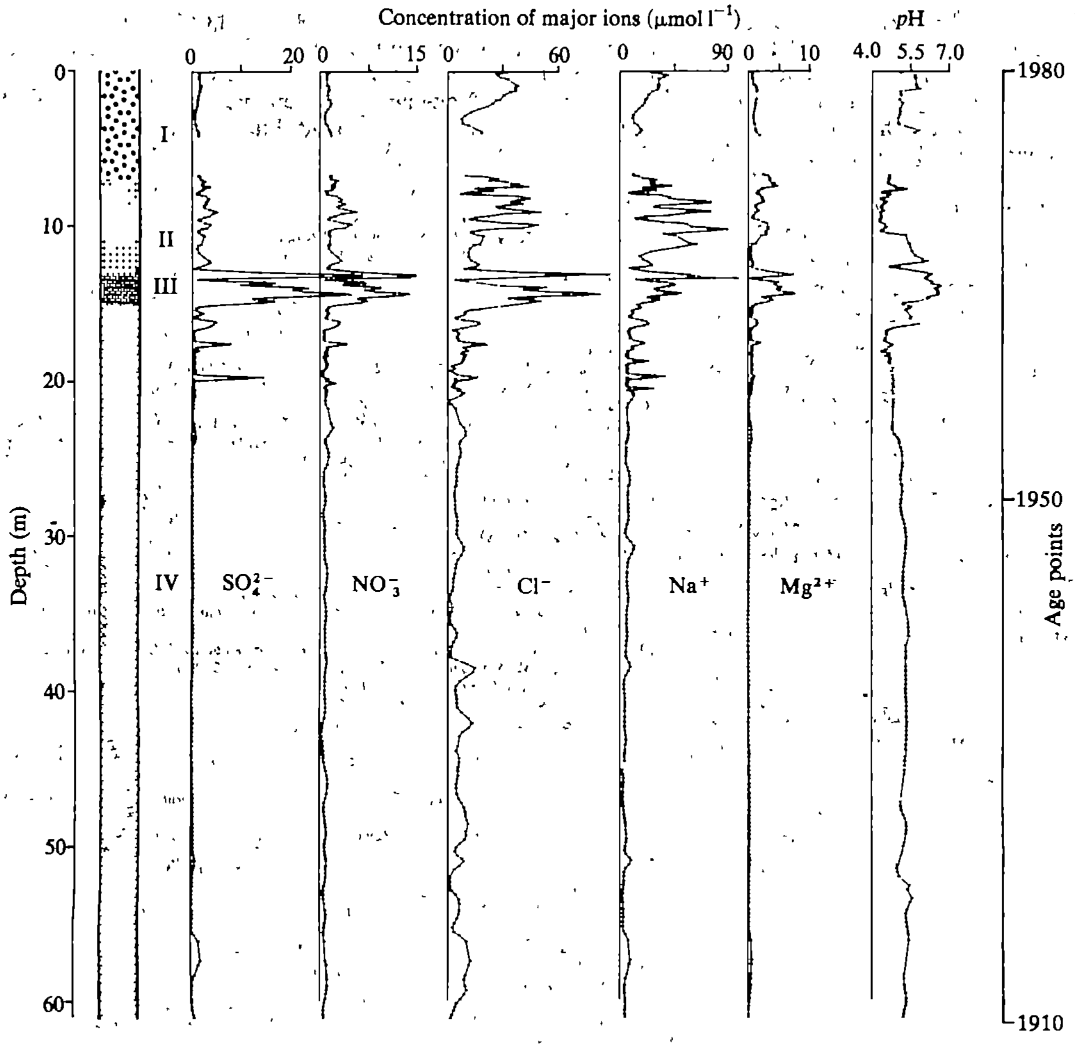

Fig. 1 Distribution of major ions down the ice core; the data have been smoothed using a 5-point binomial filter. Structure of core: I, snow and firn; II, firn and open-structured ice; III, ice with vertically-connected bubble system; IV, bubbly ice. The age points are based on climatically-reconstructed accumulations, simple flow models and tritium determinations.

An ice core, 61 m long, was recovered from the secondary summit (1,625 m ASL) of the Folgefonni Ice Cap (60°04′ N, 06°23′ E) near Bergen, Norway at the end of July 1980. The annual net accumulation at the drilling site is about 1 m water equivalent. Precipitation would be 3–4 times this amount and have approximate inorganic ion concentrations of: sodium, 100; chloride, 115; nitrate, 22; sulphate, 24; magnesium, 10; hydrogen; 35; ammonium, 15; calcium, 8 μmol l^{-1} (ref. 5). The core was retrieved using a hand auger for the upper snow and firn and a portable thermal corer[6] for the more consolidated firn and ice. The core was returned to the UK in its frozen state for analysis. About 1 m of core between 6 and 7 m was lost during the transfer from hand auger to thermal corer. The overall structure of the core was: 0–7 m snow and firn, 7–13 m firn and relatively open-structured ice, 13–15 m a zone of ice with systems of vertically interconnected bubbles, below 15 m bubbly ice (Fig. 1). The retrieved core (7-cm diameter) was cut into disks (5-cm thick). Before analysis, an outer 1-cm annulus was removed and the remaining central sections sluiced with deionized water until about half of the original volume remained. The firn sections were cut using carefully cleaned polytetrafluoroethylene (PTFE) tools and not washed with deionized water. All samples were filtered through 0.22-μm nucleopore filters. The anionic components were determined on a Dionex 12 ion chromatograph. Concentrations of the sulphate and nitrate anion were determined to better than 10% accuracy in the 1–10 μmol l^{-1} range with a detection limit of 0.1 μmol l^{-1}. Chloride levels were determined to 10% accuracy with a 0.5-μmol l^{-1} detection limit. Sodium and magnesium were determined on a Pye Unicam SP9 atomic absorption spectrometer; accuracy was ~5% and detection limits better than 0.5 μmol l^{-1}. The H^+ ion concentration was determined with a pH meter read to 0.1 pH unit. The ionic composition of the core below 20 m was more constant relative to the upper 20 m and analyses were made only on every fifth disk.

The distribution of major ions down the core is shown in Fig. 1. The few NH_4^+, Ca^{2+} and K^+ determinations available (the small volume of the ice disks precluded complete ion analysis) indicate that these ions probably provide the necessary charge for balance. The concentration of ions in the ice below 20 m are generally an order of magnitude lower than that which would be expected from the composition of the precipitation input. This is in agreement with earlier observations[7,8]. More striking is the high peak in concentrations at 12–15 m, where the ice had many vertically interconnected bubbles. The peak is most pronounced for nitrate and sulphate. Below the water table (20 m) the concentration of ions is very low, which suggests that the bulk of the inorganic solutes are removed from the ice cap. Summing the ions present above this point yields approximately: SO_4^{2-}, 0.070; NO_3^-, 0.050; Cl^-, 0.40; Na^+, 0.46; Mg^{2+}, 0.030 mol m^{-2}. These values are of the same order as a single year's input of inorganic ions for such an area of southwestern Norway, indicating that much of the ion input since the 1979 melt season had not yet (at the time of core retrieval) been removed from the ice cap, although it has been redistributed through deeper (and older) ice. The melt water percolates down through the upper open-structured layers of the ice cap each year, taking the bulk of the ionic components of that year's input with it. These reach the water table and are ultimately lost from the ice cap, eventually leaving residual concentrations of the level in the lowest 40 m of the core.

However, the percolation does not occur without redistribution among the ions. The ratios of the volume-averaged concentrations of ions in the top 3 m of the core, C_{0-3} (representing the remaining winter input), to the average ionic concentrations in the entire top 20 m of the core, C_{0-20} are: SO_4^{2-}, 0.39; NO_3^-, 0.58; Cl^-, 1.20; Na^+, 0.90; Mg^{2+}, 1.15. These values may be seen as some kind of retention index (a proportion of the ions, particularly the more mobile ones, may already have left the system and this will distort the calculated values), and suggest that some ions are eluted from the snow and firn more rapidly than others. Differential enrichment of the ions is also apparent in the vertically ducted ice zone (C_{12-15}/C_{0-20}): SO_4^{2-}, 3.8; NO_3^-, 2.5; Cl^-, 1.9; Na^+, 1.4; Mg^{2+}, 3.5. Our examination of data from snowpack studies[8-10] show similar rankings in terms of ion retention, with Cl^- and Na^+ being retained longer, NO_3^- retained for shorter periods and SO_4^{2-} and Mg^{2+} being the most mobile of the measured ions. Thus, the fractionation process (removal of the bulk of the impurities from the snowpack in the first fractions of meltwater) that has received much comment[7-10], is also a differential process, releasing some ions more quickly than others.

The average ion concentrations of various sections of the ice core are shown in Fig. 2. Note that Na^+ and Cl^- seem to be less mobile than the NO_3^- and SO_4^{2-} in the ice column. Our examination of snowpack data[8-10] also indicates the high mobility of the H^+ ions which probably therefore accompany the mobile NO_3^- and SO_4^{2-} ions as a strong acid peak appearing relatively early in the melt season. A hint of this process may be seen at the 12–15-m peak, where the SO_4^{2-} and NO_3^- concentrations are high, and the removal of each SO_4^{2-} and NO_3^- ion would lead to the associated removal of three H^+ ions. The H^+ concentration is low in this part of the core and the charge is balanced by NH_4^+ and K^+ (which, on the basis of the measurements made so far, appear to be relatively immobile). The C_{0-3}/C_{-20} value for H^+ is 0.48, confirming the high mobility of the ion, but the distribution of H^+ down the ice core does indicate the complexity of the removal process, and the effects of buffering might conceal even larger H^+ ion mobilities. If a melting season was reasonably long then we would expect the more slowly removed Na^+ and Cl^- ions to appear after the acids, making the total removal of ions equal to the input, maintaining a steady-state condition. However, short melting seasons might allow temporary retention of the less mobile constituents. The fractionation has previously been observed in snowpacks, but here we can see it occurring in a vertically extended ice core in a differential manner. It does allow us to perceive the way in which the 'acid flush' may arise from an ice cap, a situation which has some similarities with snow melt and is of obvious importance to the vernal acidification of watercourses.

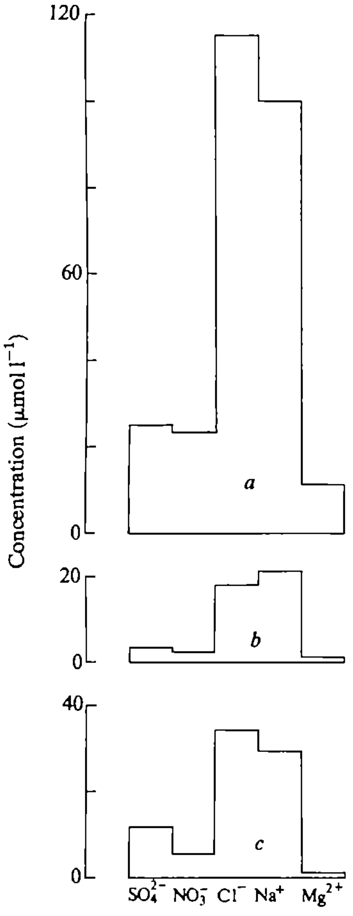

Fig. 2 *a*, Composition of precipitation input on the glacier since 1979 melt season, estimated from Norwegian Institute for Air Research data *b*, Average composition of 0–20 m zone of ice core. *c*, Average composition of 12–15 m zone of ice core.

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Stable isotopic evidence for latest Miocene sea-level fall in the Mediterranean region

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The discovery of a marked isochronous decrease of carbon isotope ratio values (~0.5–0.8 ‰ in marine carbonates at about 6.2 Myr has stimulated several speculations regarding the origin of this event[1-3]. Here we report stable isotope studies of planktonic and benthic foraminifera from the late Miocene Carmona–Dos Hermanos section in southwestern Spain which suggest that (1) the carbon shift at 6.2 Myr is directly associated with a eustatic sea-level lowering recorded in the same section; (2) the shift in $\delta^{13}C$ at 6.2 Myr and other times may be related to an increased flux of marine and terrigenous organic matter depleted in ^{13}C and eroded from the continental shelves during sea-level lowstands; and (3) the sea-level fall at 6.2 Myr triggered the rapid shift towards hypersaline conditions in the Mediterranean well before the deposition of the Messinian evaporites.

.Climatic and oceanographic events that may be closely associated in time with the decrease in $\delta^{13}C$ values include: (1) an overall cooling of surface water[4,5]; (2) an increase in bottom

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Table 1 Stable isotopic results from the Carmona–Dos Hermanos section, southwestern Spain

Sample no.	Thickness(m)	*Orbulina Universa*		*Globigerinoides obliquus*		*Cibicidoides mediocris*		*Uvigerina* sp.		*Globicassidulina subglobosa*	
		$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$
C17	410.0	−0.98	1.03	−1.85	0.82						
		−0.90	0.96	−2.06	0.64						
C12	305.0	−0.83	0.83	−2.10	0.78	−0.10	−0.87	0.48	−1.51		
		−0.80	0.72	−1.93	0.81	−0.12	−0.59				
C11	292.0	0.00	0.93			0.41	−0.35	1.24	−1.24	0.96	−0.81
C10	280.0	−0.93	2.04	−1.14	1.69	0.47	0.27	1.10	−0.83	0.89	−0.53
C9	260.0	−0.53	1.94	−1.28	1.69	0.15	0.40	0.85	−0.58	0.83	−0.05
C8	240.0	−0.73	2.19	−0.40	1.56	0.17	0.02	1.15	−0.62	1.15	0.40
C7	225.0	−0.16	1.67			−0.03	−0.23	0.63	−0.59	0.66	−0.21
C6	203.0	0.02	2.25	−1.06	1.78	0.61	0.13	0.83	−0.54	0.76	−0.11
								0.95	−0.70		
C5	184.0	−0.42	1.33			−0.14	−0.19	1.11	−0.85	0.64	−0.47
C4	165.0	−0.73	1.68			0.15	−0.12				
C3	153.0	−0.70	2.27	−1.63	1.57	−0.21	0.25	0.57	−0.58	0.44	−0.34
C2	130.0	0.08	1.99	−1.67	2.17	0.46	0.35	0.99	−0.49	0.75	−0.14
C1	110.0	0.01	1.99	−0.89	1.49	0.21	0.34	0.88	−0.54	0.63	−0.33
		−0.54	2.54								
AG3	20.0	−0.37	1.80	1.56	1.17	0.29	1.07	0.69	−0.64		
AG2	0.0	−0.57	1.44			0.05	0.65	0.69	−0.70	0.47	0.15

water circulation rates and fertility of the oceans[2]; (3) lowering of sea level[6,7]; (4) the isolation and drying up of the Mediterranean[8,9]; (5) the shoaling of the Isthmus of Panama[10], (6) an increase in biogenic silica removal in the Southern Ocean high productivity zone[11]; (7) a decrease in biogenic silica sedimentation in the eastern equatorial Pacific[12]; (8) a change over from an Atlantic carbonate compensation depth (CCD) shallower than the Pacific CCD before the shift to a deeper CCD in the Atlantic after the shift[13]; and (9) an increase in deep-sea accumulation rates[14]. The cause of the decrease in $\delta^{13}C$ values and its apparent close temporal association with these other palaeoceanograpic phenomena has not been established.

One way to decrease oceanic $\delta^{13}C$ values is to introduce isotopically light (−20 to −25‰) organic matter to the open ocean by eroding it from the continental shelves during a sea-level lowstand[2,15]. A eustatic lowering of sea level has been reported close to the time of the carbon shift[6,9,16]. However, because the carbon shift has mostly been reported from deep-sea drilled sections and eustatic sea-level lowerings are documented in shallow land-based marine sections, direct correlation has proved difficult. Our stable isotopic study of marine carbonates from a land-based marine section in southwestern Spain reveals an abrupt $\delta^{13}C$ shift which coincides with a previously recorded latest Miocene sea-level fall[6].

The Guadalquivir Basin in southwestern Spain (Fig. 1) appears to represent a fully marine equivalent of the Messinian evaporite sequence[6]. Its position in the Betic Straits region in southern Spain which acted as a gateway controlling water movements between the Atlantic Ocean and the Mediterranean Sea during the Miocene[8] and its shallow depth make it ideal for studying the relationship between sea-level events, climatic changes and Messinian events in the western Mediterranean region.

The Carmona–Dos Hermanos section consists of three units (Fig. 2). The lower 'blue marls' or 'marne azzure' are a thick sequence of marls containing abundant planktonic and some benthic foraminifera and are overlain by a band of alternating sands and marls—'sandy marls'[6]. The upper unit known as the Caliza Tosca is a coarse-grained limestone containing abundant molluscan shell fragments and a high percentage of quartz.

Berggren and Haq[6] examined the distribution of benthic foraminiferal assemblages in the Carmona section and estimated changes in bathymetry based on comparisons with the depth distribution of similar living assemblages. Below the Caliza Tosca the benthic foraminiferal fauna is relatively homogeneous, but between samples C9 and C12 (Fig. 2) there is a sequential elimination of certain species. Between samples C10 and C13 benthic foraminifera species diversity drops from 46 to 3 species. The dramatic benthic faunal changes at this location, between samples C9 and C13, have been interpreted to reflect a local sea-level fall of ~200 m due to the combined influence of long term uplift in the Betic region and more rapid global causes[6].

Carbon isotopic ratios in both planktonic and benthic foraminifera decreased dramatically by nearly 1‰ between samples C10 and C12, reflecting a change in seawater isotopic

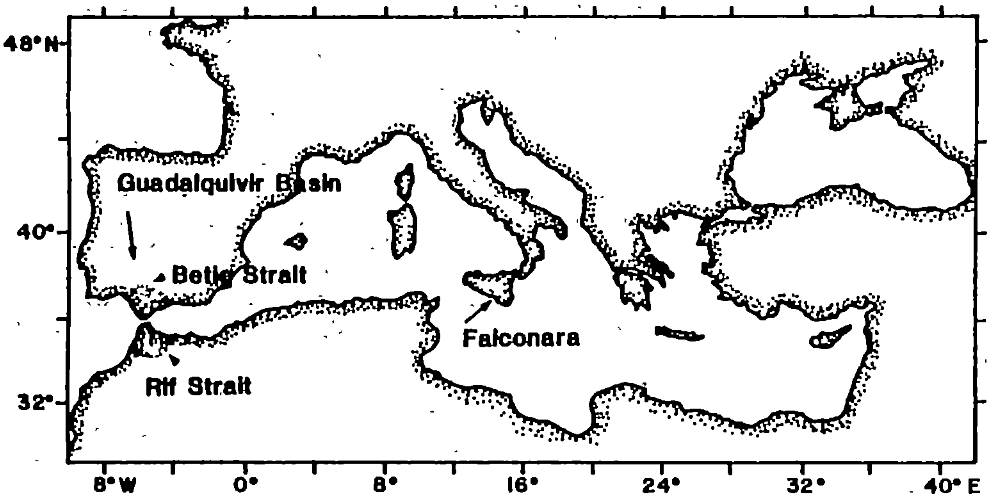

Fig. 1 Map of the Mediterranean Sea and surrounds showing the location of features referred to in the text (modified after ref. 30).

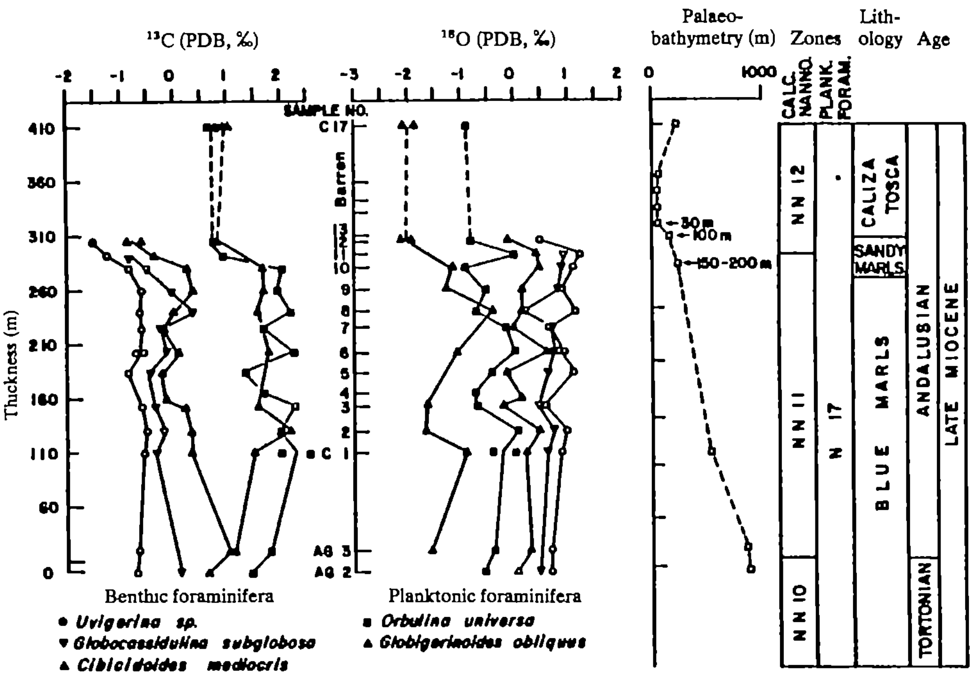

Fig. 2 Benthic and planktonic oxygen and carbon stable isotopic results (‰, PDB), palaeodepth estimates[6] and microfossil zonation schemes and lithology[6] from the late Miocene Carmona–Dos Hermanos section in southwestern Spain. Two planktonic foraminiferal species, *Orbulina universa* and *Globigerinoides obliquus* and three benthic foraminiferal species, *Uvigerina* sp., *Globocassidulina subglobosa* and *Cibicidoides mediocris*, were picked from the 250–350 μm size fraction and analysed according to standard procedures[17]. Results are presented in the notation

$$\delta‰ = [(R_{\text{sample}}/R_{\text{reference}}) - 1] \times 10^3$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{18}O/{}^{16}O$, respectively, for $\delta^{13}C$ or $\delta^{18}O$ with respect to PDB (see ref. 17).

composition (Fig. 2, Table 1). An estimate of the timing of the $\delta^{13}C$ shift in the Carmona section is critical to understanding its origin. Is it related to the latest Miocene carbon isotopic shift reported[2,3,17] in the Indian, Pacific and Atlantic Oceans? Based on the Berggren and Haq study[6] the carbon shift recorded in the Carmona section appears to occur within the lower part of the Messinian between 6.5 and 5.5 Myr. It is difficult to define the age of the $\delta^{13}C$ decrease at this location because the stratigraphical position of each biostratigraphical datum is poorly defined (±15 m) due to wide sample spacing and the reliability of each datum when applied in the Mediterranean region is questionable. The placement of the Caliza Tosca as being coeval with the Mediterranean evaporites is based on stratigraphical position and the absence of *Discoaster quinqueramus* from one sample (C12) rather than on positive evidence and is therefore also questionable[9]. The widespread Epoch 6 marker *Amaurolithus primus* found below the carbon shift in many Pacific sections is not present in the Carmona section.

The latest Miocene open ocean carbon shift is dated palaeomagnetically at ~6.2 Myr within magnetic chron 6 [6,18,20]. On the basis of biostratigraphical criteria, we cannot state that the carbon isotopic shift in the Carmona section is time-equivalent with the open ocean shift. On the other hand, the similarity in age of the isotope shifts, the fact that the shifts occur in both planktonic and benthic foraminifera simultaneously and that the magnitude of the shifts is similar, suggest that a globally synchronous geochemical event which affected both shallow and deep ocean waters is also recorded in the Mediterranean region.

Oxygen isotope results at the Carmona section reveal that the magnitude of the average difference between values from the benthic foraminifera *Uvigerina* and the shallow dwelling planktonic foraminifera *Globigerinoides obliquus* is nearly constant and >2‰ (Table 1, Fig. 2). This isotopic difference probably reflects a surface to bottom temperature contrast of 8–9 °C because the benthic foraminifera fauna (*Bulimina*, *Discorbis*, *Cibicides* and *Melonis*) suggest normal marine rather than hypersaline conditions. Also the presence of psychrospheric ostracods in the middle to late Miocene Mediterranean Sea indicate cool bottom water temperatures[21]. Similar planktonic and benthic foraminiferal $\delta^{18}O$ differences are found elsewhere in the Mediterranean[22].

One possible way of rapidly decreasing organic $\delta^{13}C$ values in the latest Miocene is by introducing oxidized organic matter or increasing the rate of flux of carbon from a major carbon reservoir to the ocean[2,15]. Such changes could be produced by increasing the oxidation of organic matter on the deep ocean sediment surface as a result of increased benthic erosion and higher oxygen content in deep waters[1,3]. However, Broecker[15] has suggested that the cycle of production, deposition and removal of continental shelf sediments during transgressions and regressions across the shelf could produce a significant change in open ocean $\delta^{13}C$ values. The shelves act as a sink for carbon during transgressions and a source during regressions, when erosion supplies previously deposited organic matter, depleted in ^{13}C, to the open ocean[2,15].

Close temporal association of the distinct global negative carbon isotopic shift and a eustatic lowering of sea level in the latest Miocene suggest that the two may be causally linked. In addition the correspondence between sea-level changes and a negative $\delta^{13}C$ shift in marine carbonates recorded at Carmona has been noted in two New Zealand land sections: Blind River and Eketahuna[18]. Many late Miocene sections record significant lowerings of sea level evidenced by a global lack of shallow water carbonates, facies changes in ostracod faunas and benthic foraminifera[9] and marine phosphorite deposits[23]. Palaeontological correlation of such events is especially difficult because of the removal of continental margin sequences by erosion, the short-time interval involved and poor micropalaeontological control in many continental margin sequences. The sea-level fall is estimated to have been between 70 and 100 m and to have occurred rapidly ($<10^4$ yr). Such rapid changes in sea level are inferred to have been caused by glacio-eustatic events. Based on oxygen isotopic analyses of marine carbonate in DSDP Site 284, Shackleton and Kennett[24] suggested that an increase in Antartic ice-volume sufficient to produce a sea-level fall between 40 and 70 m occurred in the latest Miocene. However, further isotopic analysis of many late Miocene DSDP drill sites and piston cores have found $\delta^{18}O$ changes similar in magnitude to that recorded at Site 284 throughout the late Miocene, but no permanent change in oxygen isotopic values[17,25,26]. This apparent incompatibility between stratigraphical, lithological and oxygen isotopic evidence for a latest Miocene sea-level change hinders a full understanding of late Miocene palaeoceanography. We suggest two possible mechanisms for this incompatibility: (1) the oxygen isotopic record is missing during the critical time due to unconformities and (2) large amounts of continental ice, more enriched in ^{18}O than Antarctic ice, were formed in the latest Miocene. Evidence for the first mechanism is provided by detailed biostratigraphical studies which reveal a hiatus in nearly all latest Miocene sediment recovered from the Pacific Ocean by the Deep Sea Drilling Project[27]. The second mechanism is supported by Mercer and Sutter[28] who suggest that a Northern Hemisphere ice-sheet about one-half the size of the Pleistocene Laurentide ice-sheet formed in the latest Miocene. The formation of this ice-sheet with an isotopic composition of ~ -30‰ could significantly lower sea level without dramatically raising the $\delta^{18}O$ of the oceans.

An understanding of the structure of the water column in the Betic area before the eustatic lowering of sea level and the Messinian evaporitic events allows us to place constraints on the stratigraphical position of the Caliza Tosca. Studies by Cita *et al.*[29] and Hsü *et al.*[30] indicate that the changes from open marine conditions occurred suddenly ($<10^4$ yr). There is no evidence in the Carmona section for a rapid increase in salinity to hypersaline conditions before the sea level fall. There is, however, a small (<0.5‰) gradual increase in bottom water $\delta^{18}O$ values up the section which possibly occurred as a result of the slow tectonic uplift of the Betic straits region gradually restricting bottom water return flow to the Atlantic. Thus, a eustatic lowering of sea level cut off the return flow to the Atlantic of deep waters resulting in rapid isolation of the Mediterranean Sea and causing hypersaline conditions.

We tentatively correlate the rapid lowering of sea level at Carmona with the beginning of hypersaline conditions within the Mediterranean region. A change to hypersaline conditions in the deep waters of the Mediterranean would have occurred rapidly as a result of surface water evaporation once the bottom water return flow to the Atlantic was shut off in the Betic straits. Initially, a restricted supply of North Atlantic surface waters maintained normal marine surface water salinities in the Mediterranean but eventually as the Atlantic portal closed the surface waters also became hypersaline. Total evaporation could have occurred within $\sim 10^3$ yr of final closure of the Atlantic–Mediterranean gateways. Thus, the change to hypersaline conditions in the Mediterranean probably occurred within at most, a few thousand years, of the eustatic sea-level event recorded at Carmona and therefore provides a valuable stratigraphical marker within the Mediterrean region independent of biostratigraphical criteria. Hypersaline conditions are recorded in the Tripoli (marly diatomites) of the Falconara section in Sicily[31,32], situated very near the Messinian stratotype sections at Pasquasia and Capodarso. Planktonic foraminiferal[31] and bulk carbonate[32] $\delta^{18}O$ values increase dramatically in the upper part of the Falconara section at the base of the Tripoli formation immediately following the demise of most benthic forminifera, which suggests that the bottom waters became hypersaline before the surface waters.

We suggest that a eustatic sea-level fall recorded at Carmona and the global decrease in $\delta^{13}C$ values correlate with the lowermost Tripoli Formation based on the almost simultaneous (within 5,000 yr?) change to hypersaline conditions in the Falconara section. If we are correct in assigning an age of 6.2 Myr to the sea-level event and associated carbon isotope shift then the base of the Tripoli in the Falconara section is ~6.2 Myr. If the absence of *Nitzschia miocenica* and *Thalassiosira praeconvexa* is due to ecological exclusion[33], then the Tripoli could be as old as magnetic chron 6, in good agreement with our age estimate.

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Isotopic and geochemical studies of nodules in kimberlite have implications for the lower continental crust

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Lower crustal xenoliths from the Calcutteroo kimberlitic pipes in South Australia show a surprisingly diverse range in Nd and Sr isotopic compositions and rare earth element (REE) abundances. Mafic granulite and garnet–clinopyroxenite xenoliths have measured $\varepsilon_{Nd}(0)$ values between +6.1 and −7.9, both light (L)REE enrichments and depletions, low Rb/Sr ratios (0.005–0.040) and relatively high $^{87}Sr/^{86}Sr$ ratios of from 0.70675 to 0.70919. A felsic xenolith has compositions more typical of upper crustal rocks with $\varepsilon_{Nd}(0) = -21.4$, LREE enrichments, Rb/Sr = 1.47 and $^{87}Sr/^{86}Sr = 0.85987$. An approximate correlation also exists between Sm/Nd versus $^{143}Nd/^{144}Nd$ and Rb/Sr versus $^{87}Sr/^{86}Sr$ indicating major intracrustal differentiation in the lower crust at ~2,400 Myr. This produced extremely low Rb/Sr ratios, and both relative LREE enrichments (low Sm/Nd) and depletions (high Sm/Nd). If these results are typical, then they indicate that the lower crust has higher $^{87}Sr/^{86}Sr$ and more positive $\varepsilon_{Nd}(0)$ values than previous estimates[1–5]. This may require revision of total crustal compositions and crust–mantle evolutionary models[1–5].

Although the composition of the upper continental crust is established reasonably well by direct observations, the composition of the lower crust remains uncertain. Xenoliths derived from lower crustal depths by kimberlite magmas provide one of the few direct means of sampling the lower crust, as it exists *in situ* at present. In this study, we present Nd and Sr isotopic data, together with selected trace element abundances for lower crustal xenoliths from the Calcutteroo kimberlitic pipes in South Australia. The kimberlite host rocks of the inclusions are intruded into folded, Proterozoic marine sediments[6], located in the Flinders Range, about 230 km north of Adelaide in South Australia. Measured Rb/Sr ages on phlogopites separated from kimberlitic matrices are in the range 164–174 Myr (ref. 7).

The inclusions consist of garnet clinopyroxenites, garnet granulites and felsic garnetiferous gneisses. Grain sizes vary

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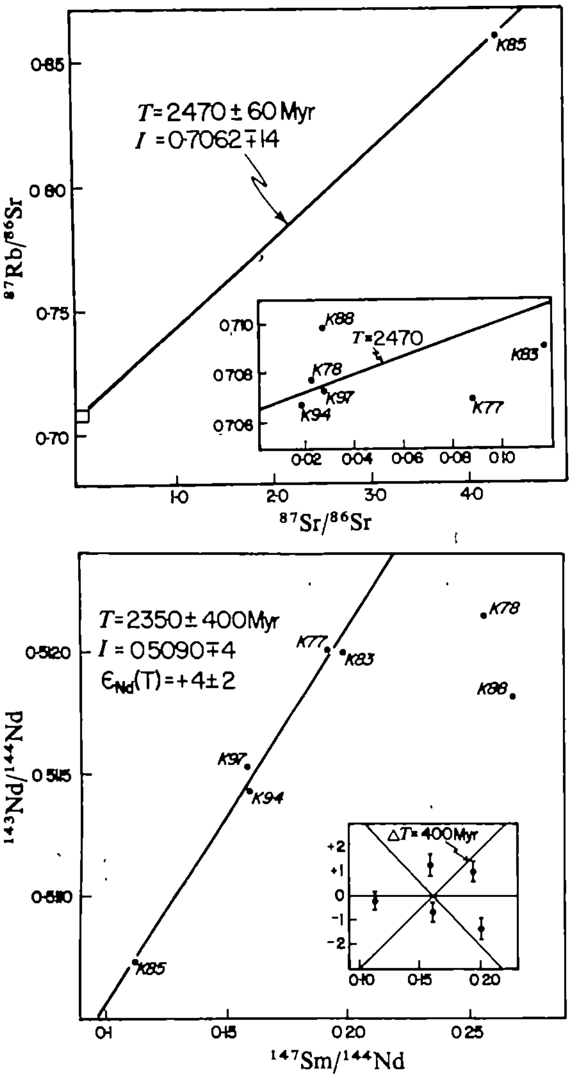

Fig. 1 *a*, Rb–Sr isochron diagram for lower crustal xenoliths from south Australia. The age is controlled by the felsic granulite K85. The low Rb/Sr samples (shown in inset) have significant deviations from the 2,470 Myr isochron but all indicate high initial $^{87}Sr/^{86}Sr$. *b*, Sm–Nd isochron diagram. A regression (excluding K78, K88) gives a similar age to Rb–Sr. Inset shows deviations from the best fit isochron in parts per 10^4. The deviant samples K78 and K88 indicate that the lower crust sampled by the kimberlite is heterogeneous in age and/or initial ratios.

from fairly uniform, microgranular (<0.5 mm) through subequant granular (0.5–1.5 mm) to porphyroblastic (<5.0 mm garnet). Some samples have a distinct mineral lineation. Phase assemblages in the basic inclusions are dominated by omphacitic diopside–salite, intermediate Mg/Fe garnet with ~20–25 mol % grossular, and plagioclase in the compositional range oligoclase–andesine (R.J.A., J.F. and B.W.C., in preparation). Minor and accessory phases consist of rutile, biotite, hastingsitic amphibole, K-rich feldspar, scapolite, quartz, apatite and sphene. The more felsic nodules have a diverse mineralogy including, in the sample analysed for Nd and Sr isotopes, almandine-rich garnet, K-rich feldspar (Ca:Na:K = 0.5:19:80.5), kyanite, quartz, rutile and biotite (Mg/Mg+ΣFe ≈ 0.62). Orthopyroxene, amphibole, sphene, scapolite and rare REE-U-rich phosphate phases are present in variable proportions in other inclusions of intermediate to felsic composition in the Calcutteroo kimberlitic pipes[8] (R.J.A. *et al.*, in preparation). Equilibration temperatures and pressures[9–12] for the inclusions are in the range 800–900 °C, 8–12 kbar equivalent to depths in the crust of the order 25–40 km. The depth of the Moho in this region is suggested to be ~35 km (ref. 13). Most inclusions are basaltic in chemistry with <51% SiO_2, and MgO in the range 7–11 wt%. Intermediate SiO_2 (~55%) and high-SiO_2 (60–68%) inclusions constitute a minor fraction of the inclusion population.

The Sm–Nd and Rb–Sr isotopic measurements are given in Table 1 and shown in Fig. 1*a*, *b*. There are correlations between measured Sm/Nd versus $^{143}Nd/^{144}Nd$ and Rb/Sr versus $^{87}Sr/^{86}Sr$ ratios (Fig. 1*a*, *b*). A regression of all the Rb–Sr isotopic data yields an age of 2,470±60 Myr and an initial $^{87}Sr/^{86}Sr$ ratio of 0.7062±14. The age is controlled solely by the high Rb/Sr sample (K85) and the initial $^{87}Sr/^{86}Sr$ by the remaining low Rb/Sr samples. A regression of the Sm–Nd isotopic data (excluding K78 and K88) yields a similar, although less precise, age of 2,350±400 Myr and an initial $^{143}Nd/^{144}Nd$ ratio of 0.5090±4 (corresponding to $\varepsilon_{Nd}(T) = +4\pm2$). The xenoliths K78 and K88 deviate markedly from this array. These latter samples also have pronounced LREE depletions (Fig. 2) thus excluding the possibility that their REE abundances and Nd isotopic compositions have been modified by contamination with the host kimberlite magma, which is strongly enriched in LREE[8]. Instead, the deviations must reflect some real heterogeneities in age and/or initial ratios of the lower crust sampled by the xenoliths.

The initial $^{87}Sr/^{86}Sr$ ratio of 0.7062±14 is substantially greater than mantle values indicating that the major chemical fractionation event registered by both isotopic systems occurred within the crust rather than as a consequence of mantle–crust differentiation processes. The high initial Sr ratio can be accounted for by a period before ~2,400 Myr of high Rb/Sr ratios within the lower crustal protoliths. For example, with an Rb/Sr ratio the same as K85 (Rb/Sr = 1.5), $^{87}Sr/^{86}Sr$ would evolve in an interval of ~80 Myr from a mantle value of 0.702 to the observed initial ratio of 0.7062. For a Rb/Sr ratio more typical of the upper crust (Rb/Sr = 0.3)[14], this interval would be increased to ~400 Myr. The initial Nd isotopic composition of $\varepsilon_{Nd}(T) = +4\pm2$ indicates derivation from a source which has had a previous history of relative LREE depletion. Although this is generally more characteristic of mantle sources[3,4,15,16], it is still compatible with a relatively short crustal residence time. For example, assuming that the initial crust was LREE enriched (similar to K85), a crustal prehistory of ~100 Myr would reduce the $\varepsilon_{Nd}(T)$ value by ~1 unit. Therefore, to account for the observed ε_{Nd} (2,400 Myr) value of +4±2, this would imply derivation from a mantle source with ε_{Nd} (2,500 Myr) = +5±2, which is still compatible with available constraints[16]. Substantially longer crustal prehistories are possible if the lower crustal protoliths had either chondritic LREE or depleted LREE abundances. This is conceivable considering the highly variable REE abundances in the xenoliths.

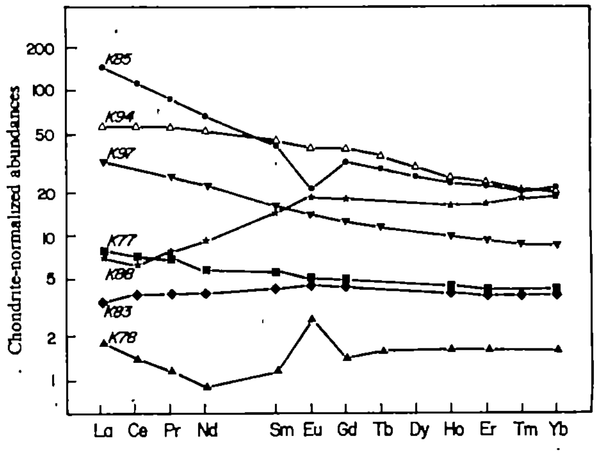

Fig. 2 REE patterns determined using spark-source mass spectrometry[24] and normalized to chondritic abundances. The lower crustal xenoliths have a diversity of patterns and only K78 has the positive Eu anomaly predicted for the lower crust[14,17] and found in the Lesotho granulite xenoliths[25].

Table 1 South Australian lower crustal xenoliths

	Samples	Sm	Nd (p.p.m.)	Rb	Sr	^{87}Rb/^{86}Sr	^{147}Sm/^{144}Nd	^{143}Nd/^{144}Nd†	^{87}Sr/^{86}Sr†	$\varepsilon_{Nd}(0)$‡
K77	gnt–cpx–plag (bio–rut–qtz)*	1.05	3.32	4.16	137.3	0.0875	0.1900	0.51201±3	0.70708±6	3.4
K78	gnt–cpx–plag (bio–rut–qtz)	0.29	0.67	1.02	135.2	0.0218	0.2565	0.51215±6	0.70770±5	6.1
K83	gnt–cpx (sc–rut–qtz)	0.95	2.90	4.37	108.1	0.117	0.1979	0.51200±2	0.70919±6	3.2
K85	gnt–kyanite–kspar–qtz (bio–rut)	8.84	47.56	169	115	4.306	0.1124	0.51074±2	0.85987±6	−21.4
K88	gnt–cpx–plag (rut–apt–amph)	1.87	4.20	1.47	155.1	0.027	0.2694	0.51182±3	0.70980±8	−0.3
K94	gnt–cpx–plag (qtz–rut–apt)	9.59	36.52	1.34	215.0	0.018	0.1588	0.51143±3	0.70675±4	−7.9
K97	gnt–cpx–plag (qtz–rut–apt–sphene)	4.19	16.01	1.78	187.7	0.0275	0.1584	0.51153±2	0.70736±5	−6.0
§	Average South Australian lower crust	3.2	11.8	7.5	180	0.12	0.16	0.51179	0.7128	−1.0

* Major phase listed first, minor and accessory phases in parentheses. gnt, garnet; cpx, clinopyroxene; plag, plagioclase; bio, biotite; qtz, quartz; rut, rutile; amp, amphibole; apt, apatite; kspar, K-rich feldspar; sc, scapolite.

† Nd and Sr isotopic compositions and abundances determined using analytical procedures described in ref. 26. Normalized to ^{146}Nd/^{142}Nd = 0.636151 and ^{86}Sr/^{88}Sr = 0.1194.

‡ $\varepsilon_{Nd}(0)=\left[\frac{(^{143}\text{Nd}/^{144}\text{Nd})}{(^{143}\text{Nd}/^{144}\text{Nd})_{\text{CHUR}}}\text{, sample }-1\right]\times 10^4$, where $(^{143}\text{Nd}/^{144}\text{Nd})_{\text{CHUR}}=0.511836$.

§ Average values calculated by weighing xenoliths according to their relative abundance.

Intracrustal melting at ~2,500 Myr has also been proposed by Taylor[17] and Taylor and McLennan[14] to account for the negative Eu anomaly observed in post-Archaean sediments. During this intracrustal melting event, it is envisaged that the melt becomes enriched in LREE and Rb, and depleted in Eu and Sr as a result of the preferential incorporation of Eu^{2+} and Sr^{2+} into residual plagioclase. Although this mechanism accounts for the low Rb and high Sr abundances, of the xenoliths, only one has a positive Eu anomaly. (In fact, K85 has a negative Eu anomaly.) Thus, at least for the samples analysed in this study, it would appear that retention of plagioclase in the lower crust has not been the major factor controlling the REE abundances. Variations in the abundance of REE enriched accessory minerals (such as allanite, zircon and apatite) could substantially influence the REE abundances in the lower crust, and perhaps compensate for the positive Eu anomaly produced by plagioclase.

The present-day Nd and Sr isotopic compositions are shown in Fig. 3, together with estimates of lower crustal compositions by other workers[1–3,5,14]. The xenoliths analysed in this study clearly differ from all other lower crustal estimates, primarily due to the high measured ^{87}Sr/^{86}Sr ratios and more positive $\varepsilon_{Nd}(0)$ values. Several workers have assumed that the relative REE abundances are the same in both the upper and lower crust[1,2,4,18]. This assumption is based on the observation that Archaean felsic granulites usually have LREE-enriched patterns similar to upper crustal patterns[18–21]. Apart from the *ad hoc* assumption that Archaean felsic granulites are representative of the present day lower crust, the significance assigned to the felsic gneisses ignores the presence in many Archaean granulite terrains (such as Scourian[5,20]) of granulites having intermediate, basic and ultramafic compositions. These latter rock types are all lower in absolute concentration of LREE, occasionally LREE-depleted, and have small positive or negative Eu anomalies. The relative proportions of these more basic rock types in these terrains is conjectural at present, but this study, as well as others on suites from kimberlitic pipes in New South Wales, Victoria, and the Colorado Plateau, USA, all suggest that basic rock types may be an important constituent of the lower crust[8,22,23].

Although reliable estimates of lower crustal compositions must await more data of the type reported here, it is, nevertheless, informative to speculate on its implications for total crustal compositions. Typical upper crust (for a mean age of 1,500 Myr)[1] has $\varepsilon_{Nd}(0) = -15$ and ^{87}Sr/^{86}Sr = 0.7220, Nd = 26 p.p.m. and Sr = 350 p.p.m. (ref. 14). Thus, total crust consisting of 1 part upper crust to 2 parts lower crust[14] has $\varepsilon_{Nd}(0) = -8.3$, ^{87}Sr/^{86}Sr = 0.7173 (for a mean age of 1,500 Myr)[1] and Nd = 16 p.p.m. and Sr = 230 p.p.m. (based on our analyses of lower crustal xenoliths). These compositions are significantly different from previous estimates for the total crust and may require

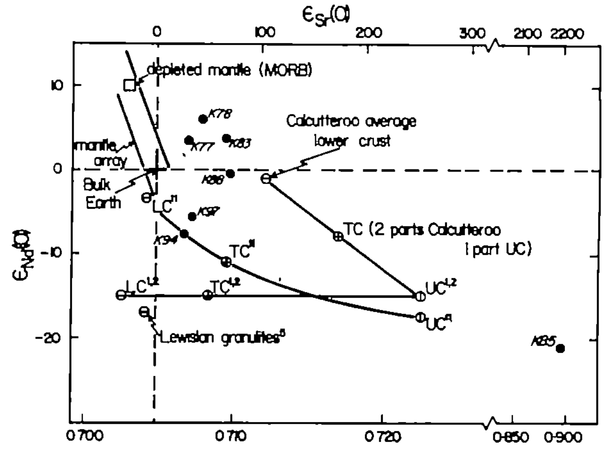

Fig. 3 Present-day Nd and Sr isotopic composition for south Australian lower crustal xenoliths (●). The xenoliths have both positive and negative $\varepsilon_{Nd}(0)$ values and have relatively radiogenic ^{87}Sr/^{86}Sr ratios. The felsic granulite K85 which has extremely high ^{87}Sr/^{86}Sr and negative $\varepsilon_{Nd}(0)$ values is a rare xenolith type. The south Australian lower crustal average is significantly different from existing models particularly those that assume that the relative LREE abundances are the same in the upper and lower crust[1–5,18]. The closest agreement is with the models of Taylor[17] and Taylor and McLennan[14] which assume intracrustal fractionation of Sm/Nd as well as Rb/Sr and thus predict more positive $\varepsilon_{Nd}(0)$ values for the lower crust compared with the upper crust. Lines connecting lower crust (LC ⊖), total crust (TC ⊕) and upper crust (UC ⏀) are calculated by assuming that the lower crust constitutes two-thirds of the total. The inferred total crust composition calculated using the lower crustal xenoliths is also substantially different from previous estimates[1,2,14]. If this is not due to Ha sampling bias, it may imply, for example, the existence of additional reservoirs to satisfy the bulk Earth constraint[1–5] of $\varepsilon_{Sr}(0) = \varepsilon_{Nd}(0) = 0$.

revision of the parameters and assumptions on which some crust–mantle evolutionary models[1–5] are based. In particular, the assumption that the total crust and depleted mantle are strictly complementary and are together equivalent to the bulk Earth[1–5] is not consistent with these results.

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Ages and implications of East Pacific Rise sulphide deposits at 21°N

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Massive sulphide deposits described as inactive hydrothermal deposits (samples CYP) along the axis of East Pacific Rise (EPR) were first discovered at 21°N, about 700 m west of the active rift[1]. This discovery was followed by that of active vents made of sulphides (samples *Alvin*) in the central part of the rift valley[2]. Due to the importance of the flux of elements presently discharging and its impact on the chemistry of the ocean, it is important to understand the duration of the phenomenon and its possible periodicity. We present here an attempt to date the fossil and the active chimneys using radioisotope methods.

In the oceanic environment, chemical fractionations exist due to differences in geochemical behaviour of the different nuclides forming the decay series of uranium and thorium. Their existence in the hydrothermal environment can be postulated.

(1) The chemical separation between uranium and thorium is well known in normal oceanic conditions, and ^{230}Th has been widely used as a chronometer for oceanic events[3–7]. This method is applicable in the range of some thousands of years to about 300,000 yr. This time span cannot be used for the active vents but may be of interest for the fossil chimneys as the age of the crust on which they lie, at 700 m from the active axis, may be estimated taking into account a mean half expansion rate of 3 cm yr^{-1} (ref. 8) as $\sim$20,000 yr (ref. 1).

Uranium and thorium isotopes have been measured by α- ray spectrometry after chemical separation; results are given in Table 1. To ascertain that no ^{230}Th enters in the sulphides at the moment of the precipitation, on 10 samples from active vents, we have checked that ^{230}Th is always under the detection limit of the apparatus (Table 2), as well as ^{232}Th, so the latter may be used to detect an eventual contamination during alteration.

On two samples from the fossil deposits, CYP 78–08–14 and CYP 78–12–40, we have analysed 11 different subsamples, 8 of them are pure sulphides, they do not contain any ^{232}Th and their ^{230}Th/^{234}U ages are from <2,700 to 6,400 yr (ref. 9), (Table 1). Three subsamples which are partly oxidized exhibit measurable concentrations of ^{232}Th and higher uranium contents. The calculated ages for these samples are 6,900, 18,000 and 36,500 yr. Although the first two do not disagree with the possible age of the basement, we discard them as being wrong due to their probable alteration. Thus, we conclude that a former period of active hydrothermal discharge took place $\sim$4,000 yr ago.

(2) The half life of ^{230}Th, 75,200 yr, precludes its use for dating the chimneys sampled during *Alvin* dives in the central part of the rift valley, so we tried to use different short-lived decay products of the uranium and thorium series.

Turekian *et al.*[10] have used, for the measurement of growth rates of giant clams from Galapagos rise, the ratios ^{228}Th/^{228}Ra; ^{228}Ra/^{226}Ra and ^{210}Pb/^{226}Ra. Those nuclides have half lives between 1,622 and 1.90 yr which seem suitable for our study, provided we know the ratios at the origin of the system. Moreover, we also tried to use the decrease of activity of ^{210}Pb ($t_{1/2}$ = 22 yr), referred to stable lead.

The activities of these nuclides have been measured by non-destructive γ-ray spectrometry, and lead was measured by atomic absorption spectrophotometry.

Table 1 U and Th isotopic analysis by α-ray spectrometry

Sample	^{232}Th (p.p.m.)	^{238}U (p.p.m.)	^{234}U/^{238}U (activities ratio)	^{230}Th (d.p.m. g^{-1})	^{230}Th/^{234}U (activities ratio)	Age (kyr)
CYP 78–08–14						
A–1	0.09±0.06	14.4±0.4	1.159±0.023	0·75±0.03	0.061±0.003	6.9±0.35
A–2 (1a)	0.40±0.09	13.1±0.5	1.139±0.037	1·7±0.1	0.153±0.011	18.0±1.4
A–2 (1b)	0.21∓0.04	8.9±0.3	1.131±0.024	2.14±0.09	0.287±0.015	36.5±2.3
A–2$_{\text{II}}$						
External layer 1	⩽0.02	1.75±0.06	1.10±0.05	0.031±0.007	0.022±0.006	2.4±0.7
Layer 2	⩽0.03	1.19±0.06	1.174±0.067	0.035±0.010	0.034±0.011	3.8±1.2
Layer 3	n.d.	1.31±0.06	1.072±0.068	0.041±0.018	0.039±0.019	4.3±2.1
Internal layer 4	⩽0.016	3.0±0.1	1.123±0.034	0.064±0.007	0.026±0.004	2.9±0.5
CYP 78–12–40						
A	⩽0.02	1.22±0.05	1.192±0.059	0.031±0.008	0.029±0.008	3.2±0.9
B External pustules	⩽0.04	0.61±0.04	1.1±0.1	⩽0.012	⩽0.024	⩽2.7
Intermediate part	⩽0.016	1.58±0.05	1.082±0.038	0.050±0.006	0.040±0.006	4.4±0.6
Internal part	⩽0.08	2.8±0.1	1.104±0.039	0.081±0.031	0.035±0.014	3.9±1.6

Errors quoted are 1 σ uncertainties based on counting statistics.

The ^{210}Pb activity has been measured in the particulate sulphides precipitating directly in the plume of an hydrothermal vent as 1.8 d.p.m. ^{210}Pb μg^{-1} of Pb (ref. 11). We consider that the lead in the sulphides constituting the walls of the vents has

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Table 2 U, Th and Pb isotopic analysis

Dive	Sample	Subsample	^{230}Th (d.p.m. g^{-1})	^{234}U (d.p.m. g^{-1})	^{238}U (p.p.m.)	Pb (p.p.m.)	^{210}Pb (d.p.m. g^{-1}) whole sample	^{210}Pb/Pb (d.p.m. μg^{-1})	Age (yr)
Alvin 1									
909	R1	A	≤0.005	3.00±0.16	3.4±0.2	910	600±105	0.66±0.11	32±6
914	R1	A	≤0.006	3.37±0.16	4.0±0.2	495	213±36	0.43±0.07	46±5
923	R7	E	≤0.006	4.63±0.16	5.3±0.2	850	281±38	0.33±0.05	54±4
Alvin 2									
978	R13	1	≤0.006	0.32±0.02	0.34±0.03	330	259±37	0.78±0.11	27±5
979	R3	1	≤0.006	0.82±0.05	1.01±0.06	3,700	1000±116	0.27±0.03	61±4
980	R5	1	≤0.005	1.15±0.05	1.45±0.07	690	360±50	0.52±0.07	40±4
981	R3	1	≤0.005	0.080±0.006	0.085±0.006	240	103±32	0.43±0.13	46±10
982	R21	1.1	≤0.006	4.16±0.21	5.1±0.3	660	519±70	0.79±0.10	26±4
		1.3	≤0.005	1.10±0.06	1.28±0.07	160	139±42	0.87±0.25	23±10
982	Chimney		≤0.004	0.38±0.02	0.48±0.02	≤60	27±24	—	—
Cyana									
CYP78.12	40B		0.050±0.006	1.26±0.04	1.58±0.05	390	28±21		

^{230}Th/^{234}U ages have not been calculated as they have no significance. The error on γ measurements of ^{210}Pb is 10 statistical errors on counting rate + an error on the efficacy of Ge–Li detector estimated as 10%. The error on atomic absorption measurement of Pb is ~5% and has been neglected.

the same origin and the same ^{210}Pb activity at the time of deposition. On nine samples, ^{210}Pb activities were large enough to allow relatively precise measurements even by γ-ray spectrometry, giving ^{210}Pb/Pb ratios from 0.87±0.25 to 0.27±0.03 (Table 2)[12].

In ambient seawater, some Pb and ^{210}Pb may exist, 1 ng per kg (ref. 13) and 20 d.p.m. per 100 kg (ref. 11) respectively; this oceanic component might change the initial ^{210}Pb/Pb ratio and also progressively contaminate the sulphides after their formation. We therefore verified that, in one of the 'old' samples (CYP78-12-40B) containing stable lead, ^{210}Pb activity was below the limit of our method (28±21 d.p.m. g^{-1}) and that on a presently forming sulphide deposit containing almost no Pb (982 chimney), ^{210}Pb is not measurable. So, unless the initial ^{210}Pb/Pb ratio measured by Finkel *et al.*[11] varies from one vent to another in the same field, or if it is different in the sulphides deposited at the vent from the initial one, which is unlikely, the ages so obtained are true ages.

Unfortunately, the samples coming from different vents were not sufficiently documented especially as regards their orientation and position in the walls of the vents, to allow the meaning of the differences in ages to be dicusssed. Either they form continuously and our values are mean values between an older part and a very recent one, and, in this case, it is a lower limit for the beginning of the event, or the chimneys took only a brief time to build and do not grow at present, and our age is the age of the beginning of the event. During the last *Cyatherm* cruise, the instantaneous growth of a chimney has been visualized (R. Hekinian, personal communication) which suggests that our second hypothesis is more valid.

^{228}Th/^{228}Ra method may be applied to samples containing radium. As they do not contain any thorium when they formed, then any measurable ^{228}Th must have formed as a decay product of ^{228}Ra. Due to the relatively short half lives of the two nuclides, the equilibrium value of the activity ratio, 1.4, is obtained after about 15 yr. During this time, ^{228}Ra being unsupported decreases with its half life of 5.75 yr while ^{228}Th increases with its half life of 1.9 yr.

Only two of our samples exhibited ^{228}Th and ^{228}Ra activities measurable by γ-ray spectrometry (Table 3) for which the equilibrium value of the ratio is obtained, giving for the two samples ages >15 yr, which is in accordance with the 46±5 and 54±4 yr found with ^{210}Pb.

The last suitable ratio is ^{228}Ra/^{226}Ra. In such a young system, ^{226}Ra may be considered as stable while ^{228}Ra decreases. The problem is then to know exactly the initial value of the ratio. We may hypothesize that the two nuclides are in equilibrium with their parents ^{238}U and ^{232}Th respectively in the basalt and that they are not differentiated during leaching and precipitation. These hypotheses are quite reasonable as there is no reason to have an isotopic fractionation. The problem is then to know the ^{232}Th/^{238}U ratio of the basalts. The weight ratio is given as 2 for mid-oceanic ridge basalts, on average[14], which gives an activity ratio of 0.65. For the only two samples in which radium is present (Table 3) the measured ratios lead to ages of 16 and 12 yr, lower than the ages found with ^{210}Pb.

Recent measurements[15] have shown that tholeitic basalts from 21° N EPR have a higher ^{232}Th content, leading to a mean activity ratio of 1.13, the ages so obtained are respectively 21 and 17 yr. Moreover, in young basaltic rocks, the uranium series may be out of equilibrium, particularily ^{230}Th may be depleted which would imply a higher initial ^{228}Ra/^{226}Ra ratio. Anyway, these ages are too young compared with ^{210}Pb ages. But, due to the uncertainties in the initial ^{228}Ra/^{226}Ra, to the lack of sensitivity of γ rays measurements and to the possible higher mobility of radium isotopes than of lead isotopes, we prefer, until new measurements on well documented samples are obtained, to consider that ^{210}Pb ages are the most probable. It must be pointed out that such low ^{226}Ra activity compared with ^{210}Pb activity have no appreciable influence on ^{210}Pb/Pb ages.

Table 3 Th and Ra isotopic analyses by γ-ray spectrometry

Sample	^{226}Ra (d.p.m. g^{-1})	^{228}Ra (d.p.m. g^{-1})	^{228}Th (d.p.m. g^{-1})	^{228}Th/^{228}Ra	^{228}Ra/^{226}Ra
Alvin 1					
914 R1 A	46±4.7	4.2±0.7	6.2±0.9	1.48±0.32	0.091±0.018
923 R7 E	15±1.6	2.3±0.5	3.8±0.7	1.65±0.50	0.15±0.04

Error is 1σ statistical error on counting rate + an error on the efficacy of Ge–Li detector estimated equal to 10%. ^{226}Ra has been measured through ^{214}Bi; ^{228}Ra has been measured through ^{228}Ac; ^{228}Th has been measured through ^{208}Tl.

We conclude that: (1) These dating methods work and their precision may be improved using radiochemical separation followed by α and β countings. (2) There are two well defined systems, one which formed about 4,000 yr ago and another which is active at present. If such systems cannot develop far from the active axis, it shows that during the past 4,000 yr, the spreading rate has been as high as 15 cm yr^{-1}, and not 3 cm yr^{-1}. (3) The active system probably lasts <100 yr, but >20 yr, which is in relatively good accordance with the values calculated in ref. 16 from heat flow theoretical calculations.

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Carbon geodynamic cycle

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The systematic use of coupled isotopic tracers has improved our understanding of many problems such as the geochemical structure and evolution of the mantle. However, some fundamental questions remain[1–6]; for example, does sediment injection occur in the mantle[4,5,7], modifying composition and combining the internal and external geochemical cycles? Recently ^{3}He data from the South Atlantic have been used to suggest that the sediment injection phenomenon may be important on a local scale[8]. Here, using carbon data only, we show that sediment injection in the mantle must occur. This conclusion was reached after comparison between the integrated flux coming from the mantle and the budget of the exogeneous reservoir of carbon.

The value of the isotopic composition of carbon in the mantle was confusing for a long time. Carbonatites and most diamonds yielded values of δ^{13}C between −9‰ and −2‰ PDB (refs 9–12) whereas basalts yielded values between −25 and −30‰ (refs 12–14) (Fig. 1). Although they exist for a variety of acid residues from continental alkali rocks down to −30‰ (ref. 13), we are not aware of δ^{13}C lower than −26.5‰ for MORB tholeiites[15]. The difference between these values is of the same order of magnitude as that between reduced and oxidized forms of carbon (coal and carbonates) present at the Earth's surface—in low temperature conditions where carbon isotopic fractionation is important[16]. The results for basalts are rather surprising considering that they are formed at high temperature and could imply that the ^{13}C/^{12}C of the mantle is very heterogeneous. This situation has recently been clarified[14,15,17]. The CO_2–liquid melt carbon isotopic fractionation has been measured experimentally[15] and varies from 4.5 to 4‰ between 1,180 and 1,280 °C. Thus, if a basalt of initial δ^{13}C around −7 reaches the surface after losing CO_2, the latter is enriched in the heavy isotope and the basalt contains residual carbon of much lower δ^{13}C.

Both the carbon isotope compositions of several fractions of oceanic tholeiites[15] and of the peculiar types of tholeiites forming the popping rocks dredged on the Atlantic ridge[14] are compatible with this mechanism.

The quantitative model suggested for this outgassing is based on the calculation of the fraction of degassed carbon (relative to the original amount). Using a Rayleigh[14,15] distillation law, a relation between the isotopic compositions of the residual basalt (δ) and of the initial mantle (δ_0) can be written as

$$\delta - \delta_0 = 1{,}000(\alpha - 1)\ln f$$

with α the fractionation factor and f the carbon fraction remaining in the basalt.

Using the experimental values of $\delta_0 = -7$ and $1{,}000(\alpha - 1) = 4.5$ one may calculate a series of values for f corresponding to the different values of δ^{13}C for basalts. The commonly accepted value of $\delta^{13}\text{C} = -26‰$ corresponds to $f = 0.014$. With carbon contents of the rocks between 100 and 150 p.p.m. (ref. 14), this corresponds to an initial content of 6,800–10,200 p.p.m. In fact, δ^{13}C of residual carbon are not necessarily as low as −26‰ and distillation effects do not necessarily follow a Rayleigh model. A greater range of initial contents, of 2,000–10,000 p.p.m., is indicated by recent results[18].

One may now look at the rate of degassing per year. The thickness of the basalt layer in the oceanic crust is 2 km whereas the total thickness of the crust (basalt + gabbro) which was initially liquid, is 5 km. One may consider either that all the liquid has been degassed, directly or through intense hydrothermal circulation at ridges, or only the basaltic layer. This corresponds to two extreme hypotheses.

Considering a constant average spreading rate of 4 cm yr^{-1}, we get:

$$\underset{\substack{\text{(Length}\\ \text{of}\\ \text{ridge)}}}{\begin{matrix}6\times10^{9}\times\\ \text{cm}\end{matrix}} \quad \underset{\text{(Spreading)}}{\begin{matrix}(4\times2)\\ \text{cm yr}^{-1}\end{matrix}} \times \underset{\substack{\text{(Thickness}\\ \text{of}\\ \text{material}\\ \text{involved)}}}{\begin{matrix}2\times10^{5}\\ \text{cm}\\ \text{or}\\ 5\times10^{5}\\ \text{cm}\end{matrix}} \times \underset{\substack{\text{(Density}\\ \text{of}\\ \text{basalt)}}}{\begin{matrix}3\\ \text{g cm}^{-3}\end{matrix}} \times \underset{\substack{\text{(Carbon}\\ \text{concen-}\\ \text{tration}\\ \text{degassed)}}}{\begin{matrix}0.01\\ \text{or}\\ 0.002\end{matrix}} \quad \begin{matrix}7.2\times10^{14}\\ \text{g yr}^{-1}\\ =\\ 0.58\times10^{14}\\ \text{g yr}^{-1}\end{matrix}$$

The total mass of sediments at the Earth's surface is 32×10^{23} g, 15% of which are carbonates. This amount of carbonates corresponds to 5.75×10^{22} g of carbon[16,17].

The mass of the continental crust is 2.4×10^{25} g (ref. 19). Considering a complete granito-gneissic composition and using an average content of 300 p.p.m. carbon for these rocks[20], the granito-gneissic continental crust corresponds to 7.2×10^{21} g which is one hundredth of that present in the sediments and is thus negligible. However, considering the existence of metamorphic carbonated series which are omitted from this calculation, we must add $\sim1\times10^{22}$ g.

The maximum total carbon now present at the Earth's surface is 8×10^{22} g (the amount of carbon in the atmosphere and the oceans is negligible in this context). This gives lifetimes of carbon in the external cycle of $1.1\text{–}14\times10^{8}$ yr. (External is the cycle outside the solid Earth and includes hydrosphere, atmosphere, lithosphere and sediment, but internal includes cycles inside the Earth.) However, the extreme values which cumulate the uncertainties on initial contents and thickness of

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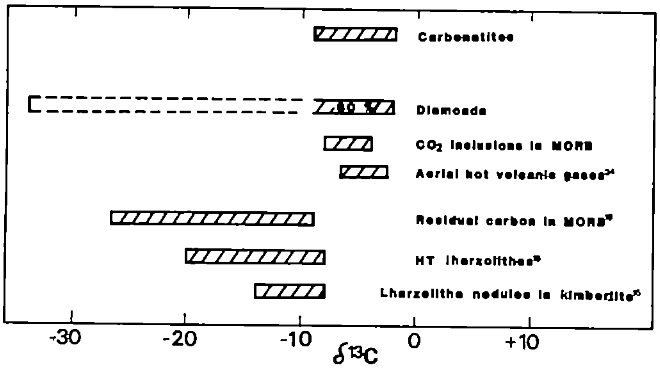

Fig. 1 The different ranges of values of $\delta^{13}C$ for various mantellic materials.

$$\delta^{13}C=\frac{(^{13}C/^{12}C)_{\text{sample}}-(^{13}C/^{12}C)_{\text{standard}}}{(^{13}C/^{12}C)_{\text{standard}}}\times 10^3$$

Standard is the PDB limestone. Results are from refs 12, 15, 18, 34, 35.

material involved are probably over-pessimistic, and the use of the mean rate value (3.9×10^{14} g yr^{-1}) which gives a lifetime of 2×10^8 yr is probably more realistic. Another way of expressing this result is to measure how much carbon has been degassed during 4×10^9 yr thus gaining information on the internal history of the Earth. Using a steady-state hypothesis, we get a mean value of 1.8×10^{24}, which represents 22 times the carbon now contained in the external parts of the planet.

We have considered a steady-state regime for the degassing during geological times but specialists agree that the latter decreased following weakening of the convection throughout this period.

One way of reconciling our observations is to admit that the exogenous cycle does not represent a reservoir of accumulation but of exchange. The carbon would thus be injected in the mantle at subduction zones, within the sediments.

Many objections have been made to the idea. How could light and soft sediments sink into a dense, hard and hot mantle?

The development of deep-sea drilling projects JOIDES and IPOD showed that some subduction zones did not have accretion prisms[21–23] and that generally their tectonic regime was of extension type. Using all types of geophysical and geological information, Uyeda and Kanamori[24] have classified the subduction zones into two types: Chile and Mariana type. For the latter, an extension occurs behind the subduction zone and then sediment injection is a possible mechanism. This process seems the most likely when considering a dynamic process for the subsidence instead of a forced injection of the subducting plate. This injection is not uniform, as shown by the JOIDES drills; some subduction zones, because of their strong distension, allow a fast injection (Central America, Marianas) whereas others with weaker distension produce accretion prisms (Caribbean, Java)[24,25]. The variable intensity of the subduction processes and the distribution of subduction zone may create important geographical heterogeneities in the mantle.

Part of these sediments contaminates the arc volcanism as demonstrated by Kay[26], but an important part sinks into the mantle. When considering a 500–1,000-m-thick layer of pelagic sediments of average density 2.5, the same spreading characteristics used for ridges, a content of 30% carbonates and 1.5% organic carbon, we get $2.8–5.6\times10^{14}$ g of injected carbon. This corresponds to the value already obtained for degassing, by a completely independent calculation.

Therefore, we may conclude that in the present conditions, the exogenous–endogenous cycle in the mantle is a steady state. Using the mean value of 4×10^{14} g for the exchange flux in both directions the lifetime of carbon in the exogenous cycle is $\tau_{ex}=2\times10^8$ yr. Let us now calculate the residence time in the endogenous cycle. Considering that the convection system related to the plate motion only affects the upper mantle, as shown by isotopic and fluid mechanics studies, the mass of mantle material involved is 1×10^{27} g. The carbon content of peridotites, for which $\delta^{13}C$ ranges from −12 to −7 (that is, primitive values), averages 400 p.p.m. (ref. 27). This corresponds to a carbon content of 4×10^{23} g for the upper mantle. The residence time of carbon in the upper mantle is thus $\tau=1\times10^9$ yr, five times longer than in the exogenous cycle.

These very short residence times are useful for explaining the present steady state. When calculating the residence time of the oceanic lithosphere in the mantle, we get $\tau=3.7\times10^9$ yr (ref. 7). Thus, the residence time of carbon in the mantle is shorter and we must admit a much greater mobility for this element. This can be explained either by the fact that carbon is in the form of fluid (CO_2) for which the rate of transfer is large relative to the diffusion coefficient in solids, or in the form of atomic carbon, in defects, which Freund[28] has shown to be very mobile.

The mass injection of carbonates also explains the presence of CO_2 in the mantle, and the evidence[29] for CO_2 has been used elsewhere[30–33] in petrological considerations. If the geodynamical behaviour of water in the mantle is similar to that of CO_2 and particularly its residence time, we may deduce important geochemical consequences: the H_2O/CO_2 ratio in the upper mantle could be equal to the chemical ratio during injection. The carbon and water contents of injected sediments average 4.5 and 3% respectively, so that the contents in water and carbon introduced through hydrothermal circulation in the

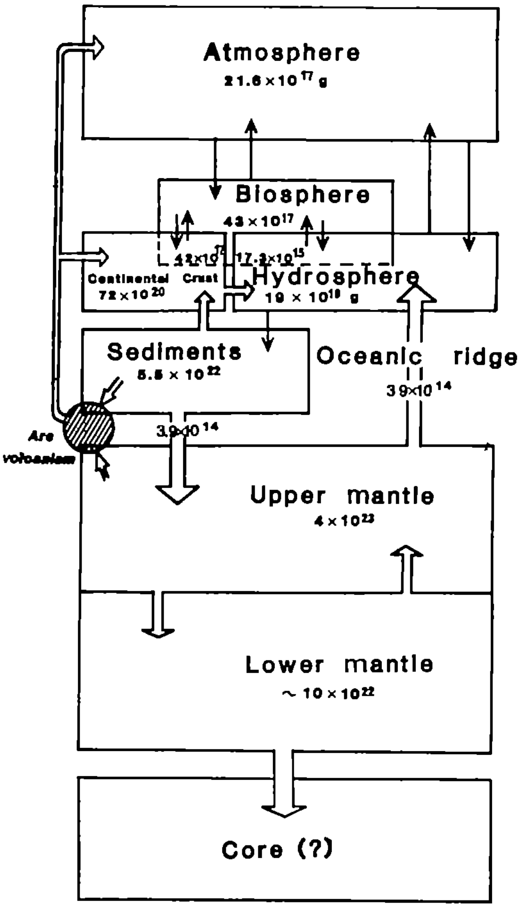

Fig. 2 Box model showing the different reservoirs for carbon and their mutual relationship. As our main interest is the exchange between exogenic and endogenic cycles, we have simplified the exogenic cycle. The masses of carbon in the reservoirs are expressed in grammes, those for the flux of exchange are in grammes per yr.

oceanic crust are 2 and 0.2% respectively. Considering a 2-km thick basaltic layer, we get 8.6×10^{14} g of water and a H_2O/CO_2 ratio in the mantle of 1.8.

We have neglected an important gas loss at volcanic arc areas—an acceptable approximation if considering a steady state. However, in future we will have to take this arc volcanism into consideration. In the steady-state hypothesis, we have the following equation for flux

$$F_{\text{ridge}}+F_{\text{arc}}=F_{\text{sediment}}$$

but the error on F_{sediment} and F_{ridge} is much too large and we cannot directly measure F_{arc}. No account has been taken for the moment of possible but unknown exchange between upper and lower mantle. Figure 2 shows the relationship proposed between these various reservoirs.

We have demonstrated that the mantle is degassing large amounts of CO_2 and H_2O. The study of isotopic composition of rare gases such as xenon and argon showed that the Earth has been suddenly degassed for ~95% in its first 50 Myr (ref. 6). If, as generally assumed, the carbon were present in the form of CO_2 and CH_4 in the atmosphere one may consider that carbon has been expelled from the mantle during this period. Thus, the exogenous reservoir of carbon was five times larger at this time than it is at present, and consequently the CO_2 content of the upper mantle much lower.

The sediment recycling began re-equilibrating both reservoirs as soon as carbonates formed in the primitive ocean and then the content of carbon in the mantle tends to increase when the content of CO_2 in the exogenic cycle decreases. At present, it is difficult to quantify such an evolution.

We point out the different history for the various mantle gases. For some of them, which are not carried heavily by sediments or subducted slabs, like probably Xe or Kr, their origins are residual after mantle outgassed. For some others, like CO_2, they are completely recycled. In this respect it would be interesting to study the behaviour of N_2 or Cl for which recent studies seem to indicate an interesting recycling history. This comparative volatile geochemistry would enable us to reconstruct the history of the atmosphere, and also of sediment recycling and mantle history.

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Reversed magnetic polarity at an early Lower Palaeolithic site in Central Italy

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Isernia La Pineta central Italy is an extensive open-air archaeological site of early Lower Palaeolithic aspect. Radiometric and palaeomagnetic dating place it in the Matuyama reversed epoch, hence before 0.73 Myr. Its early date, stratified context, abundant stone tool industry of choppers and flakes and rich faunal remains make it one of the most important localities for the question of the earliest human colonization of Europe. The archaeological horizons (Fig. 1) were discovered during recent road-building operations at 4 m below surface near the city of Isernia (Upper Volturno Basin, Province of Molise; see Fig. 2). Excavations in 1979 and 1980 carried out in two areas (sectors I and II; Fig. 2) revealed dense scatters of limestone choppers, flint flakes and disarticulated bones of large mammals. Similar occurrences have been found elsewhere in the valley. Previous accounts[1,2] are revised herein.

The archaeological levels are contained in fluvial and fluviolacustrine sediments pertaining to the principal fill of the Isernia Basin (Fig. 3)[3]. A lacustrine facies, evident at the base of this unit, ends in the north-east of the basin with banks of travertine often several metres thick. The principal fill covers depressions in a Pliocene erosional surface which retained some gradient as late as the early Pleistocene. Tectonic movements

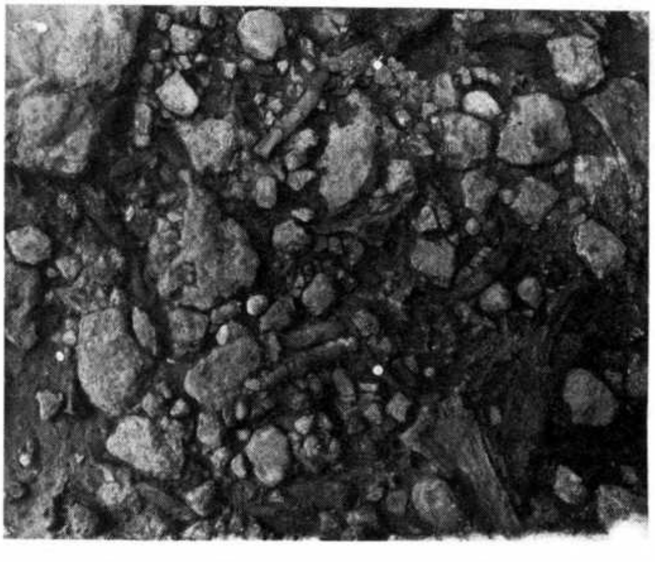

Fig. 1 Isernia La Pineta. Portion of archaeological horizon, sector I.

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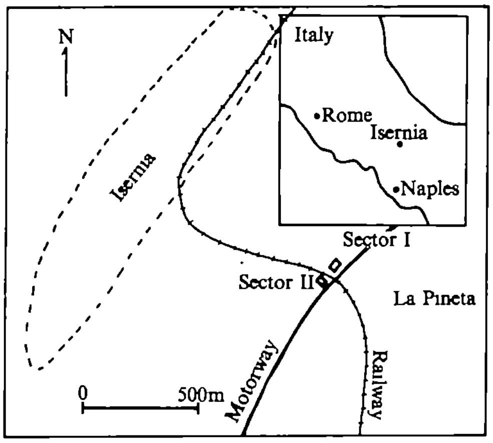


Fig. 2 Study area location map.

connected with vulcanism disrupted this surface in the middle Pleistocene, causing a considerable increase in gradient and the deposition of coarse fluvial sediments.

Numerous beds of tuff are intercalated within fluvial layers of the principal fill[4]. Grey tuffs, poor in pumice and sanidine, are found even in the lowermost lacustrine series. At higher elevations in the series tuffs are mostly brown in colour; they are partly redeposited, but all are rich in sanidine and contain crystals of biotite and pumice fragments up to 1 cm in diameter. It is thought that the volcanic activity responsible for the deposition of these tuffs took place at the same time as that of the nearby Rocca Monfina system.

Important neotectonic movements developed in the Isernia Basin, following Appenninic and anti-Appenninic directrices. Their most obvious effects consist of impressive stream capture and fault movement, involving surface rocks and/or Pleistocene deposits.

The Palaeolithic levels under consideration are sandwiched between the latest episodes of lacustrine sedimentation and the earliest fluvial deposits. The lower of the two archaeological layers encountered in sector I rests directly on a bed of travertine which shows signs of pedogenesis at its upper surface. A mantle of fluviatile muds, on which the upper occupation surface lies, covers the travertine. The upper archaeological surface was buried by sediments directly tied to an emission of tuff. The physical condition of the volcanic minerals, mainly pyroxenes and sanidines, excludes the possibility of significant fluvial transport. Fluvial sands and gravels were subsequently deposited, intercalated with tuffs and palaeosols.

From the geostratigraphical standpoint the archaeological levels seem to represent brief events, whereas the sedimentary facies in which they are included were deposited over a longer time interval.

Recent studies suggest that the Rocca Monfina volcano, whose activity had a strong influence on the Isernia basin, was active from ~1.54 to 0.34 Myr (ref. 5). K–Ar dates obtained by the Amsterdam laboratory[6] indicate that the first major phase of tuff deposition in the Isernia/Upper Volturno basin took place ~0.87–0.84 Myr.

A particularly distinctive feature of the latest depositional phase of the Isernia basin principal fill was a blanket of yellow tuff, found throughout the basin[4]. Two samples of sanidines from the yellow tuff in the Isernia La Pineta series, dated by the University of Rome laboratory, gave ages of 0.55 ± 0.05 and 0.47 ± 0.05 Myr (Fig. 3*B*), not significantly different.

A sample of the sediments immediately overlying the upper archaeological level in sector 1 gave an age of 0.73 ± 0.04 Myr. This date can be regarded as dating the archaeological level itself, as these sediments, directly tied to volcanic activity, buried the archaeological surface in a very short time. In view of the indications that the fluvial material from the top of the archaeological horizon up to tuff II was deposited rapidly, this age is also in stratigraphical agreement with the date of 0.73 ± 0.07 Myr obtained by the Amsterdam laboratory. The sample (biotite) that gave the latter date was collected from a tuff in the Belfiore series (Fig. 3*B*) ~ 1 km north of the Isernia La Pineta section. The tuff is stratigraphically equivalent to tuff II at the latter section[6].

Palaeomagnetic dating was done at the University of Pittsburgh. Oriented samples were measured in a cryogenic magnetometer. Polarities range from 'clearly reversed' to 'possibly reversed' and 'indeterminate' (because of heavy present field overprinting). No sample showed unambiguous normal polarity, so it is probable that deposition of the sediments sampled, and thus the archaeological layers as well, date to the Matuyama reversed epoch. There are no minor reversed episodes or excursions within the earlier half of the Brunhes to which the sample polarities could alternatively be ascribed. An attempt is being made to locate the Matuyama–Brunhes interface in the series.

The age indicated by palaeomagnetism and K–Ar (late early Pleistocene) is older than the age customarily ascribed to the Isernia fauna, and leads to doubts regarding the age of that faunal assemblage.

The archaeological layers have yielded a large sample of faunal remains, still in process of study. The materials from sector I, which are the better preserved, are being restored *in situ* and studied in detail as conservation is completed and they are removed.

The material from sector II consists of skeletal remains of large mammals, principally bison, bear, elephant, rhinoceros and hippopotamus. The parts represented, all fragmentary and mostly incomplete, include vertebrae, ribs, scapulae, pelves, crania, and epiphyses of long bones. The only complete parts are teeth, second and third phalanges, carpals and tarsals—all parts that lack marrow.

The faunal material from sector I, mostly complete bones, is represented predominantly by crania of bison and a significant number of cranial and dental remains of the other large mammals. The long bones are mainly of elephants.

The large number of bones represent a small number of mammalian species. Most frequent is bison (*Bison* cf. *schoetensacki* Freudenberg), followed by rhinoceros (*Dicerorhinus* cf. *hemitoechus* Falconer), elephant (*Elephas antiquus* Falconer), and bear (*Ursus deningeri* von Reichenau); *Hippopotamus* sp. cf. *amphibius* L., *Sus scrofa* and *Hemitragus* sp. are rare. Cervids are represented by possibly a single *Dama* individual.

A few rodent bones were recovered from water-sifted fine sediments in the fauna-bearing layers. Because of scarcity and poor preservation, only a few isolated teeth could be identified (Fig. 4). The species represented are *Clethrionomys* sp., *Microtus arvalinus* Hinton, *Pitymys* cf. *arvaloides* Hinton, *Arvicola* cf. *mosbachensis* Schmindtgen, *Pliomys episcopalis* Meheley, and *Pliomys lenki* Heller.

The megafaunal assemblage has hitherto been ascribed to the middle Pleistocene. The micromammals, however, although scanty, confirm the indications from palaeomagnetic and radiometric dating that human activities at the site took place in the late early Pleistocene.

The low frequency of cervids—ordinarily the commonest large mammals encountered in middle Pleistocene faunas of the Mediterranean basin—calls for an attempt at explanation. The situation might be attributable to selection on the part of the hunters for animals with a greater meat yield, but the picture obtained from study of other Pleistocene sites is one in which hominids hunt all ungulates present in a region, with the result that the fauna reflects, if imperfectly, the real faunal population. Only carnivores are normally under-represented in human cultural deposits. Bears are an exception, but even their proportion is considerably lower than that of ungulates and other animals.

The tentative conclusion is that a specific climatic type with two seasons is represented at Isernia La Pineta: a brief wet

A

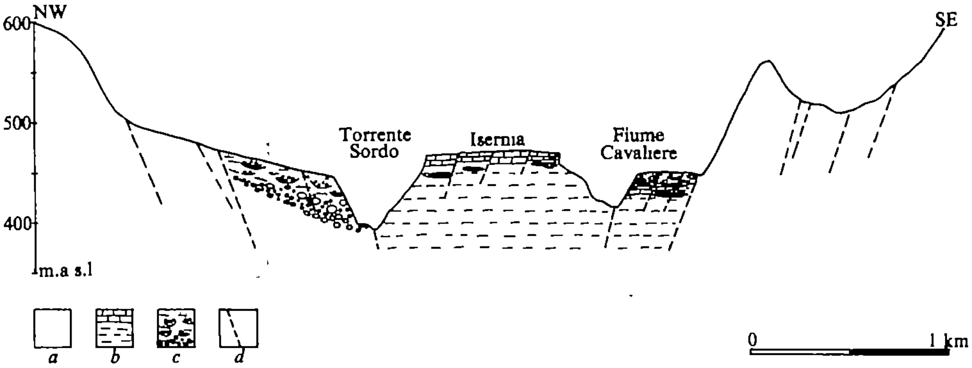
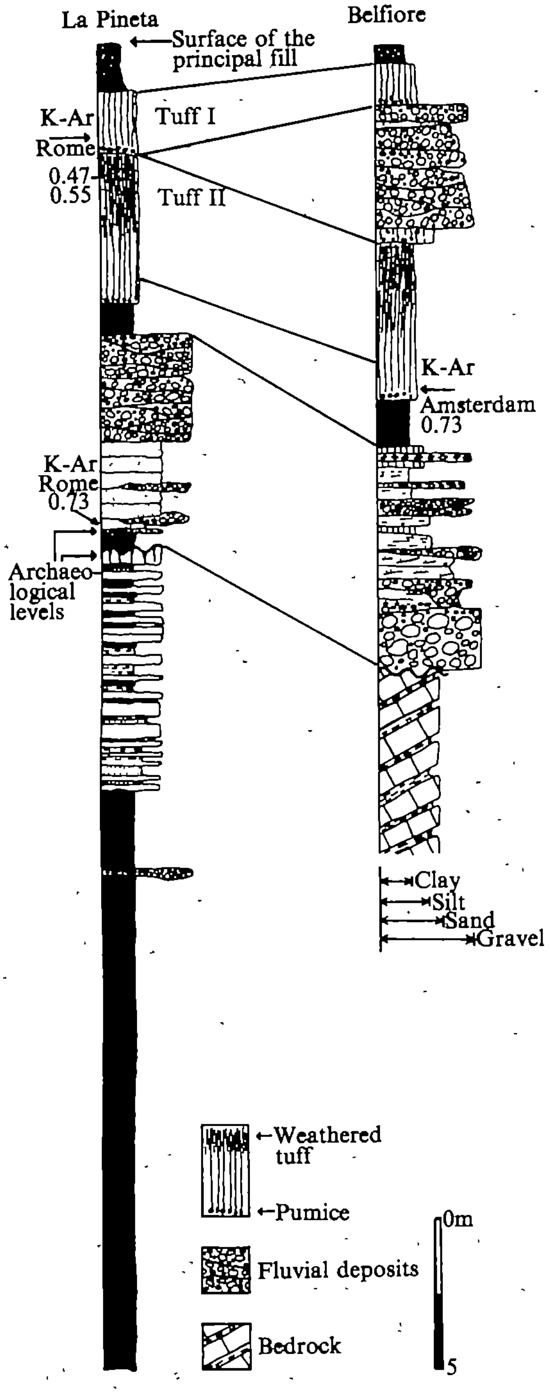


Fig. 3 *A*, geological profile at Isernia. *a*, Miocene–Eocene rocks; *b*, lacustrine sediments and travertines; *c*, fluvial sediments; *d*, faults. *B*, stratigraphy at Isernia.

B



season and a much longer arid season, resulting in an open steppe or grassland-steppe environment, well suited to a variety of pachyderm and bison species.

A continental molluscan fauna was studied from a clayey layer $\sim$12 m below the upper archaeological horizon[7]. The species found are *Lymnaea truncatula*, *Vertigo pygmaea*, *Vertigo moulinsiana*, *Pupilla muscorum*, *Vallonia pulchella* and *Succinea oblonga*. Molluscan associations of this kind characterize both middle and late early Pleistocene cold phases of Central Europe.

In sector II, an archaeological horizon of $\sim$70 m^2 was excavated. It had undergone weathering, which advanced the partial decalcification of the bones. Areas were encountered with heavy concentrations of lithic artefacts, most of small size. Tools include denticulates, notches, 'Tayacian'-like points (including some with dihedral ventral surfaces) and rare scrapers. There was a near absence of choppers. The faunal remains associated with the lithic scatters include a few fragments of diaphyses, mostly of small size and poorly preserved, and dental remains of bison, rhinoceros, elephant and bear.

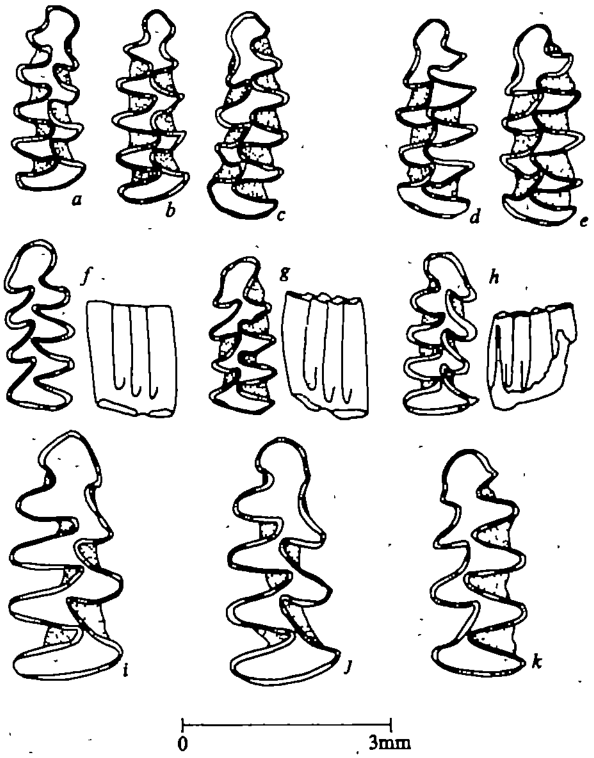


Fig. 4 Isernia La Pineta. First lower molars of micromammals (magnification ×10). *a–c*, *Pitymys* cf. *arvaloides*; *d–e*, *Microtus arvalinus*; *f–h*, *Clethrionomys* sp.; *i–k*, *Arvicola* cf. *mosbachensis*.

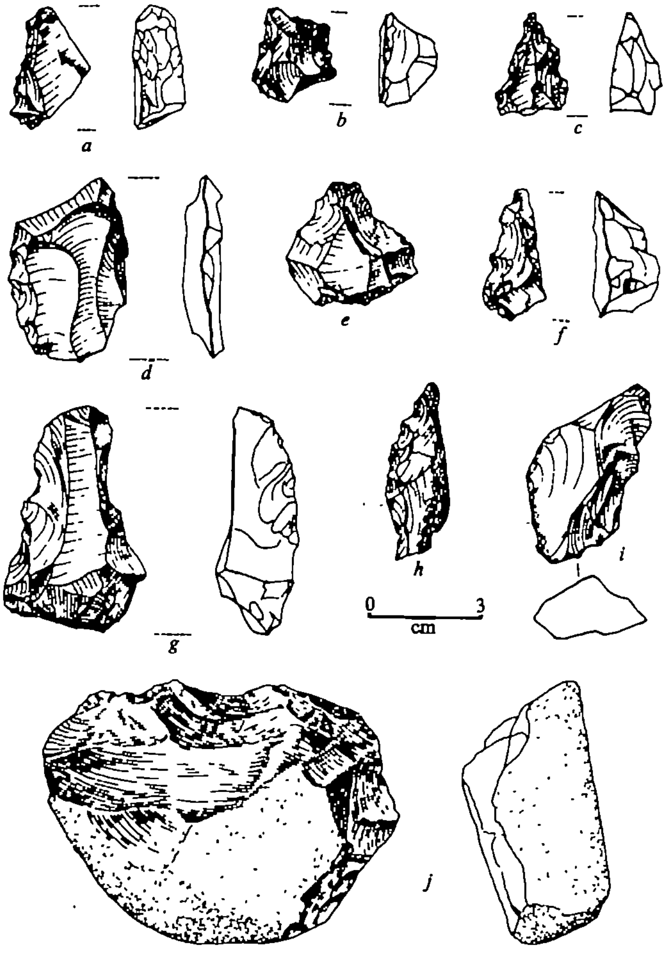


Fig. 5 Isernia La Pineta. Artefacts: *a–i*, denticulate tools on flint flakes; *j*, limestone chopper.

About 53 m^2 have been exposed in sector I. Several large blocks of limestone and travertine lay on the archaeological surface, which was covered with the bones of large mammals, with stone tools scattered among them. The disposition of the stone blocks and faunal remains strongly suggests human intervention. The faunal parts present are highly non-random. There were some 20 crania of bison; crania, mandibles and teeth of rhinoceros, hippopotamus and bear; tusks, molars and long bones of elephants. The stone tools—numbering in the thousands—can be divided into two major categories (Fig. 5). Choppers, nearly all of limestone river pebbles or cobbles, were highest in frequency. The other category comprises flake tools of flint, all much smaller than the choppers. Some can be identified as denticulates; there were only a few scrapers. Many were utilized but unmodified.

In 1980, an earlier cultural horizon was encountered in sector I beneath the one just described, separated from it by ~50 cm of culturally sterile deposits. This earlier layer, considerably lower in density of cultural materials than the one overlying it, rested on a bank of travertine. The lithic industry and faunal remains were in all respects similar to those from the upper layer.

It is not easy to find comparable sites for discussion. The few that have been ascribed to a similar age either yielded poor or ambiguous artefact inventories or do not clearly indicate a human presence. Others are surface scatters or lack secure provenience in some other way. Some of them are discussed elsewhere[8,9]. The more secure of them at least support the existence of a non-Acheulian industry in the late early Pleistocene.

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A ziphodont mesosuchian crocodile from the Eocene of Algeria and its implications for vertebrate dispersal

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Fossils from a newly discovered, probably middle Eocene locality in southern Algeria provide the first evidence of an African terrestrial mesosuchian crocodilian with a ziphodont dentition (that is, with compressed, serrated teeth). Ziphodont mesosuchians were previously known mainly from South America and Europe, but have not been reported from North America. Several other groups of land vertebrates show a disjunct distribution in the Palaeogene, with representatives in Europe and South America, but no certain record in North America, where they should occur if, as has been suggested, dispersal had taken place from South America to Europe across North America. The discovery of a ziphodont mesosuchian in Algeria is the first tangible evidence that may support the alternative hypothesis of faunal connections between South America and Europe via Africa.

The crocodilian material described here was collected from the newly discovered El Kohol locality, near Brezina in southwestern Algeria, which is being excavated by a team from the universities of Paris and Oran under the direction of J. J. Jaeger. The continental vertebrate fauna, which is still being studied, includes a lungfish, a crocodilian, and various mammals (hyracoids and a form related to *Barytherium*). It seems to indicate an age older than the lower levels of the Fayum, possibly middle Eocene.

The crocodilians are represented at El Kohol by lower jaw fragments, isolated teeth, vertebrae and a fibula. The vertebrae are amphicoelous, which is indicative of the archaic suborder Mesosuchia. The fibula is elongated and slender, which may be related to a mainly terrestrial way of life. The teeth are especially interesting. They are definitely compressed laterally (although not as much as in some other ziphodont crocodilians) and bear two sharp, finely serrated carinae; the more posterior ones are rather blunt, with a finely wrinkled enamel, while the anterior ones are more pointed, with less distinct wrinkles. Two lower jaw fragments are available: one is an almost complete

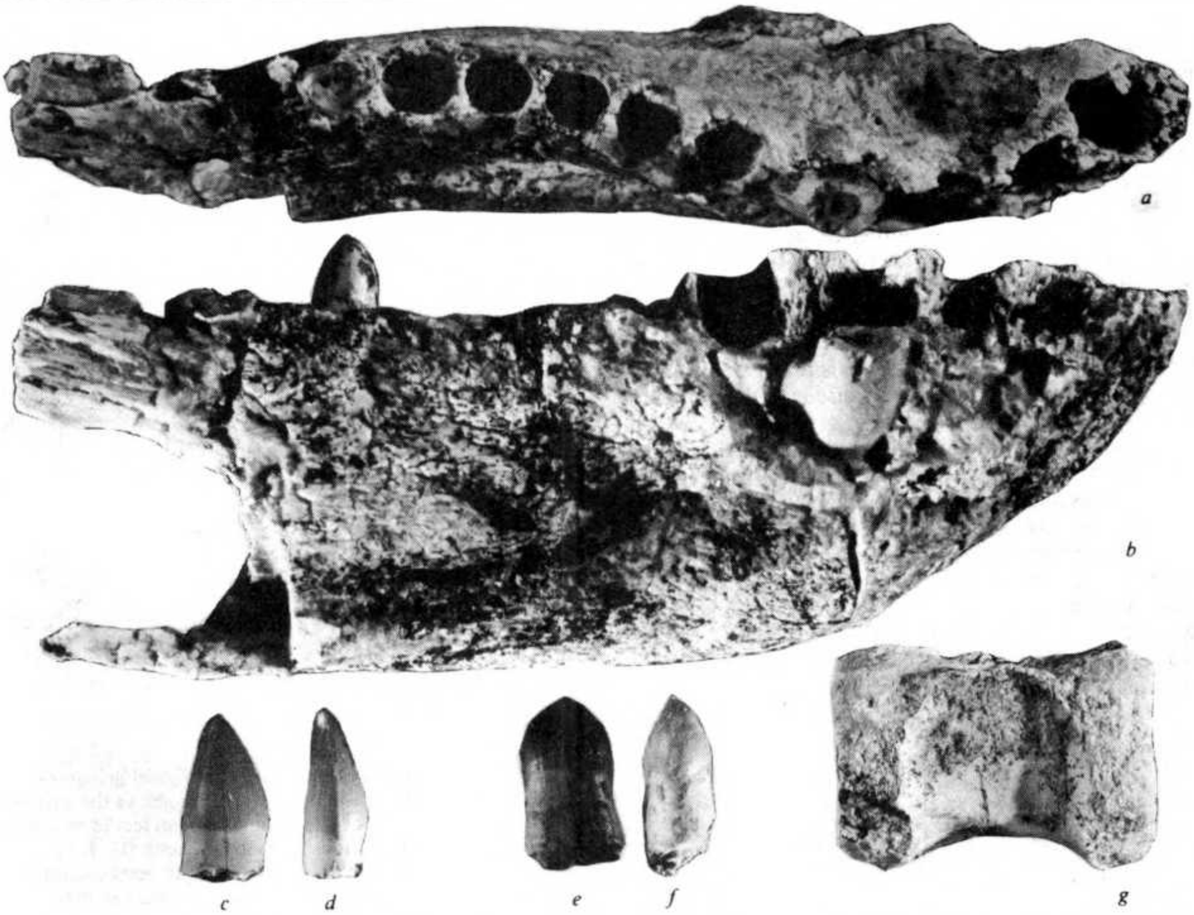

Fig. 1 Remains of a ziphodont mesosuchian crocodile from the Eocene of El Kohol, Algeria. *a*, *b*, Right dentary in dorsal (*a*) and lateral (*b*) views; *c*, *d*, two views of an isolated anterior tooth, showing lateral compression; *e*, *f*, two views of a posterior tooth, showing a blunt apex; g, lateral view of an amphicoelous caudal vertebra. ×1. (Photographs by C. Abrial.)

right dentary, still bearing one complete tooth similar to the isolated ones described above, the other is the back part of a left mandibular ramus with the articular region. The dentary is remarkably deep and laterally compressed; its lateral surface shows a marked concavity. It bears 12 alveoli arranged in a sinuous row; the fourth alveolus is the largest. The dentary symphysis reached the level of the fifth alveolus, and the splenials too apparently took part in the symphysis. The articular region of the other jaw fragment is remarkable mainly because the surangular takes part in the jaw articulation.

The affinities of this ziphodont mesosuchian, which undoubtedly represents a new taxon, are still difficult to determine with complete accuracy. The depth of the dentary is reminiscent of the sebecosuchian *Baurusuchus*, a ziphodont mesosuchian from the Upper Cretaceous of Brazil[1]. The articular region of the mandible is similar to that of *Sebecus*, a Tertiary sebecosuchian from South America[2], but it also closely resembles that of *Trematochampsa*, a non-ziphodont mesosuchian from the Upper Cretaceous of Niger and Madagascar[3,4]. Although they are compressed and more distinctly serrated, the teeth too are somewhat reminiscent of the Trematochampsidae. Comparison with the few known ziphodont mesosuchians from Europe is difficult. The lower jaw of the Algerian form is quite unlike that of *Bergisuchus dietrichbergi* from the middle Eocene of Germany[5]. In *Iberosuchus macrodon*[6], from the Eocene of Portugal and Spain, only the upper jaw is known. It should also be mentioned that the vertebrae from El Kohol are similar to those of a still undescribed ziphodont mesosuchian from the late Eocene of La Livinière (Hérault, southern France).

The biogeographical interpretation of the crocodilian from El Kohol depends on the phylogenetic relationships between the ziphodont mesosuchians of South America, Africa and Europe. Independent evolution of the European forms is rather unlikely, for lack of suitable local ancestors. On the basis of the available material, however, the hypothesis that ziphodont mesosuchians appeared independently in South America and in Africa cannot be completely dismissed. The alternative hypothesis is that all three geographical groups are closely related, or, in other words, share a common ziphodont ancestry. Although the details of the phylogenetic history of these crocodilians and their systematic expression have still to be worked out, this more parsimonious interpretation of the record does not seem to be definitely contradicted by anatomical or chronological data. Pending future discoveries, it can be accepted as a working hypothesis that the South American Sebecosuchia, the El Kohol crocodilian, and the ziphodont mesosuchians from the European Eocene are closely related forms and not the products of convergent evolution. If this were the case the origin of the European forms would be of great interest.

In recent years, an increasing number of land vertebrates with South American affinities (that is, belonging to groups otherwise known mainly from South America) have been found in the European Eocene, some of which are unknown in the Cretaceous and early Tertiary of North America. Besides the ziphodont mesosuchians, such groups with a 'South American-European' distribution include amphibians (Ceratophryinae)[7], land birds (Phorusracidae)[8], and anteaters (Myrmecophagidae)[9]. A possible explanation for this peculiar distribution is that these groups originated in South America, and then spread to North America by way of a Central American land connection in the late Cretaceous, finally to reach Europe via the North Atlantic lands[6,7,10,11]. Other land vertebrates, such as several groups of snakes[12] and the didelphine marsupials[13], are recorded from the Palaeogene of North America and probably went through such a biogeographical history. However, the main problem about accepting a similar situation for the ziphodont mesosuchians, the Ceratophryinae, the Phorusracidae and the Myrmecophagidae is that those groups have not been reported from the rich and fairly well-known late Cretaceous and Palaeogene fossil localities of North America. It has recently been suggested by C. Mourer-Chauviré[14] that these land vertebrates may have reached Europe from Africa, after differentiating in Gondwana, either before or after South America became separated from Africa (in the latter case, dispersal across the Atlantic may have taken place along the Walvis-Rio Grande rises, which seem to have been subaerial in the late Cretaceous[7]). Evidence for faunal exchanges between Africa and Laurasia in the late Cretaceous and Palaeogene is still scanty, but does exist[14]; the recently discovered continental vertebrate fauna from the Palaeocene of Morocco (which, incidentally, does not indicate affinities with South America) is one of the most important elements in this connection[15]. However, there was hitherto no fossil evidence for the presence of the 'South American' groups of the European Eocene on the African continent. The discovery of a ziphodont mesosuchian in the Eocene of Algeria now seems to provide the first evidence that some of these land vertebrates may indeed have reached Europe from Africa.

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Competition between female relatives in a matrilocal mammal

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Evolutionary theory predicts that in sexual organisms the average parents should invest equally in their male and female progeny[1] except where related males compete with each other to mate, when selection may favour parents that produce a female-biased sex ratio[2–4]. It has recently been suggested that in species where daughters adopt home ranges overlapping those of their mothers while sons disperse, competition for resources between female siblings may select for mothers who produce male-biased sex ratios[5]. We show here that in a mammal living in matrilocal, non-territorial groups—the red deer (*Cervus elaphus*)—the presence of resident female relatives depresses the reproductive success of adult females, providing a possible explanation of the apparent bias in parental investment towards male offspring in this species[6].

Red deer hinds on the Isle of Rhum (Scotland) occupy home ranges of around 2 km^2 but spend most of their time within core areas of less than half this size (Fig. 1). Sons disperse from their mother's core area between the ages of 2 and 4 yr while daughters typically adopt core areas that overlap those of their mothers and older sisters. As a consequence of variation in mortality, reproductive success and the proportion of male calves produced, the size of these matrilocal groups ranges over 2–12 breeding hinds. Related females associate with each other throughout their lives, though the core areas of different matrilocal groups overlap extensively and hinds commonly form parties consisting both of relatives and of members of other matrilocal groups.

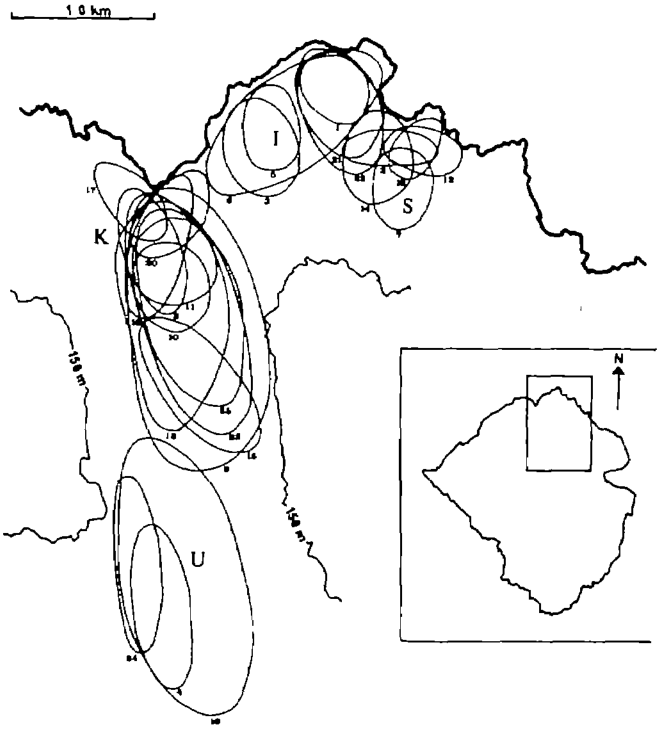

Fig. 1 Core areas of 25 different matrilocal groups of red deer hinds on the Isle of Rhum. Each ellipse shows the smallest area accounting for 65% of all sightings of members of that matrilocal group. The four divisions of the study area (U, K, I and S) are also shown. Numbers on ellipses identify individual groups and do not reflect the number of animals in them.

Records of the reproduction of 43 individually recognizable hinds obtained between 1971 and 1979 showed that hinds belonging to matrilocal groups of above average size were less successful breeders than members of groups below average size (see Table 1*a*), an effect which recurred within all four divisions of our study area (Fig. 2). To check that the negative relationship between group size and reproductive success was not a consequence of any association between the size of matrilocal groups and the number of other deer using their range, we regressed our measures of the mean number of calves reared per year by individual hinds on the number of related hinds seen feeding in their core areas (*a*) and the number of unrelated hinds seen feeding in their core areas (*b*) over a 4-yr period. Reproductive success was only related to the first of these two values (*a*: $r = -0.343$, $t_{38} = 2.249$, $P < 0.05$; *b*: $r = 0.091$, $t_{38} = 0.503$, not significant (NS)). Moreover, the extent to which a group's size increased between the first and second halves of the study period was correlated with the extent to which the reproductive success of its members fell ($r_s = 0.360$, $t_{23} = 1.853$, $P < 0.05$, one-tailed).

The reduction in reproductive success among members of large groups was partly a result of an increase in the age at first breeding (only 47% of their members conceived for the first time as 2-yr-olds compared with 79% of members of small groups), partly of lower fecundity rates over the remainder of their lifespan and partly of increased calf mortality (see Table 1). The most likely cause of the reduction in the reproductive performance of females was increased competition for food between group members: the effects of population density on reproductive performance and survival are pronounced in this

Table 1 Results of studies showing competition between female relatives in red deer

a, Reproductive performance	Large groups	Small groups	
Median no. of calves surviving to 1 yr old reared per yr	0.500 (22)	0.675 (20)	$z = 2.594, P < 0.01$
Median no. of calves born per yr	0.695 (16)	0.847 (16)	$U = 71.5, P < 0.05$
% Male calves dying in first yr of life	36.4 (118)	16.2 (117)	$G = 12.60$, d.f. = 1, $P < 0.001$
% Female calves dying in first yr of life	22.1 (95)	24.1 (87)	NS
% Males dying as yearlings	22.7 (75)	6.5 (98)	$G = 10.19$, d.f. = 1, $P < 0.01$
% Females dying as yearlings	9.1 (75)	9.3 (66)	NS
Birth sex ratio	127:100 $n = 123$	140:100 $n = 142$	NS
b, Kidney fat index	Males	Females	
Calves	1.5	1.6	$n = 26, 24$
Yearlings	1.6	2.0	$n = 38, 36$
2 Yr olds	1.9	3.5	$n = 67, 51$

a, Reproductive performance of hinds belonging to large versus small matrilocal groups (all *P* values two-tailed). A large group was one above the median size for the relevant division of the study area (see Fig. 1), a small group one that was below this size (sample sizes are given in parentheses). *b*, Kidney fat indices (weight of kidneys and perinephric fat divided by weight of kidneys) for calves and yearlings shot in winter in central Scotland (from ref. 9).

population and the frequency of aggressive interactions between hinds was greatest in those parts of the study area where population density was highest[7].

The size of a mother's group influenced the survival of her sons but had little or no effect on the survival of her daughters (Table 1*a*)—although both sons and daughters up to 1 yr old used the same food sources as their adult female relatives[7]. Feeding competition between male offspring and their female relatives was probably the principal cause of the increased mortality of young males in large groups as individuals that died had no more resident male relatives than those that survived (median test $G = 1.495$, d.f. = 1, NS). Reduced survival in conditions of food shortage in adolescent males compared with females of the same age is common among dimorphic mammals[7–13]. One contributory factor is probably the associated tendency for young males to lay down less body fat than females (see Table 1*b*). This sex difference may have evolved because the lifetime reproductive success of males is more strongly influenced by their adult body size than that of females[7], adult size is closely related to early growth and selection may consequently favour the increased allocation of nutritional resources by young males to muscular and skeletal growth instead of to the insurance policy provided by fat reserves[7,17].

We previously[6] demonstrated that in the same population hinds invested more heavily in their sons than their daughters before weaning: male calves sucked more frequently and mothers that had reared sons were less likely to calve the following year and, if they did so, calved later than mothers who had reared daughters. Despite the increased costs of producing male offspring and their higher mortality, the sex ratio of calves at weaning showed a consistent though non-significant bias towards males (118 male:100 female, $n = 347$) and we argued that our results were difficult to reconcile with Fisher's theory that parents should divide their total investment equally between their male and female progeny[1].

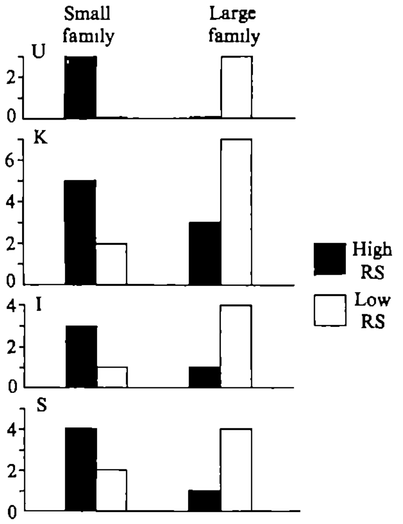

Fig. 2 Number of hinds belonging to large versus small matrilocal groups whose reproductive success (mean number of calves reared per year) are above versus below the average for the division of the study area where their home range was located (see Fig. 1). A large group was one above the median size for the relevant division of the study area, a small group one below this size.

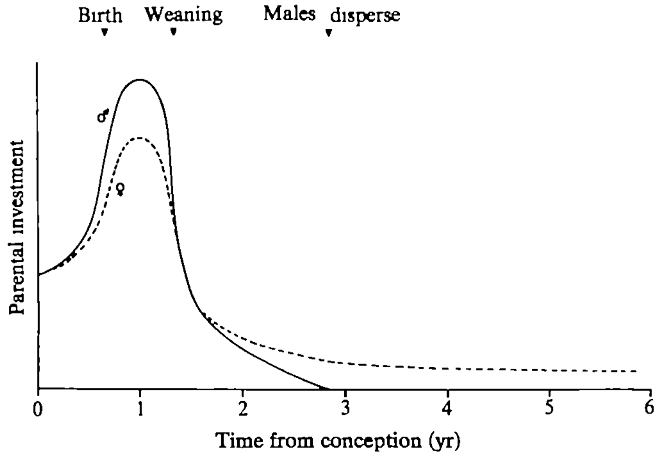

Fig. 3 Hypothetical plot of the changes in the distribution of parental investment[14] by red deer hinds to their male and female calves.

The results described here provide a possible resolution to this problem. Although sons may be more costly to rear than daughters, they disperse widely through the population and competition between adult male siblings either for food or for mates is uncommon. In contrast, daughters adopt home ranges overlapping those of their mothers and compete with each other as the size of the matrilocal unit grows.

The situation can, perhaps, be most easily viewed from the standpoint of the mother, who invests[14] more heavily in her sons than her daughters before weaning but more heavily in her daughters than her sons afterwards (see Fig. 3). This need not imply that mothers treat their daughters differently from other conspecifics (although related hinds do, in fact, tolerate each other in close proximity while feeding[7,15]) because in this context all costs of producing daughters should be considered as forms of investment. The temporal change in the bias of investment is likely to increase the mother's fitness, for our research indicates that the lifetime reproductive success of males is more strongly influenced by their body size and their early growth rates (see above) than by differences in the quality of the home range they occupy as adults, while the reverse is true of females[6,7].

Competition among female relatives for access to local resources[5] is likely to be widespread among mammals because males commonly disperse from their natal area while females remain in the locality where they are born[16]. A recent survey of the available literature shows that significantly male-biased fetal and birth sex ratios are substantially more common than female-biased ones in mammals[17]. However, a possible alternative explanation is that male-biased birth sex ratios have been favoured because they compensate for increased pre-weaning mortality among males[1] which may only occur in populations close to carrying capacity. Unfortunately, insufficient data are available to determine whether male-biased sex ratios are confined to species where daughters seldom disperse from their natal area, as the theory of local resource competition would predict.

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Structure of physiologically classified neurones in the kitten dorsal lateral geniculate nucleus

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The mammalian forebrain, including the dorsal lateral geniculate nucleus (LGN$_d$) and the visual cortex, continues both structural and functional development postnatally and is therefore a useful model for the study of developmental processes in the central nervous system (CNS). We report here the first description and comparison of the structural development of individual, functionally identified neurones in the mammalian forebrain. This comparison is made for the three main cell groups of the central visual pathways (W-, X- and Y-cells), in the neonate and the adult. In the adult, these three classes of neurones have different characteristic electrophysiological properties[1-9] and relay information in parallel about different features of a visual scene[8,9] from the retina through the LGN$_d$ to the visual cortex. In addition, each of the three functional cell types has a characteristic structure in the adult[10,11]. By injection of the enzyme marker substance, horseradish peroxidase, into electrophysiologically identified neurones, the present study demonstrates that each of these functional classes of neurones also has a characteristic morphology in the neonate (in the LGN$_d$ of kitten 3–4 postnatal weeks of age). However, striking differences in the rates of maturation are seen. The W-cells are already mature at this age. The X-cells are the least developed. Surprisingly, some Y-cells are mature. Due to the susceptibility of Y-cells to an abnormal visual environment during development[12-18], they had previously been thought to be slower to mature.

Kittens were anaesthetized with 1.5% halothane in a 1:1 mixture of $N_2O:O_2$ for all surgical procedures. All wound margins were infiltrated with 1.0% lidocaine. The kittens were maintained on a 70:30 mixture of $N_2O:O_2$ during the experiment. When an increase in heart rate occurred (above 175 beats per min) 1% halothane was administered for 5–10 min until heart rate returned to normal. Electrophysiological data were not taken during halothane administration. Respiratory control, P_{CO_2} regulation, temperature control, intravenous administration of paralytic solutions, refractions, placement of stimulating electrodes and preparation of recording micropipettes filled with horseradish peroxidase (HRP) have all been described previously[10] for adult cats. The only difference for the kitten experiments was a reduced rate of administration and concentration of paralytic (12.0 mg per kg per h gallamine triethiodide and 0.5 mg per kg per h (+)tubocurarine at 2 ml per h).

Neurones in the LGN$_d$ were initially characterized by extracellular recording. The following physiological parameters were determined for each cell: (1) the position and size of the receptive field, (2) the response type to a stationary stimulus (excitatory response to light onset or offset), (3) the regularity of the response to moving targets, (4) the strength of the receptive field surround, (5) the regularity of the response to repeated presentation of a stationary stimulus to the receptive field centre, (6) the linearity of spatial summation within the receptive field (using a sinusoidally modulated grating pattern on a cathode ray tube at an appropriately high spatial frequency[4,5,8,10]), (7) ocular dominance, and (8) the latency of response to electrical stimulation of the optic chiasm. The cell was then penetrated with the micropipette, and the receptive field properties were rechecked while recording intracellulary[10]. HRP was then injected into the cell iontophoretically. Only one cell was injected in a single electrode penetration and no more than three cells were injected in an LGN$_d$. The kittens were then deeply anaesthetized with pentobarbital and perfused transcardially with a phosphate-buffered 2.5% gluteraldehyde: 1.0% paraformaldehyde fixative 2–10 h after the injections. Tissue preparation and reaction with 3-3′-diaminobenzidine (DAB) were as previously described[10]. In some cases, the Cobolt intensified DAB reaction[19] was used.

Eighteen neurones in the 3–4-week-old kittens were characterized both physiologically and structurally (seven W-cells, seven X-like cells and four Y-cells). Of the seven W-cells (all located in the C-laminae of the LGN$_d$), all had morphological features similar to those of the adult. Five of these seven kitten W-cells were highly responsive to moving targets. They responded in a regular fashion to repeated visual stimuli, with little decline in the vigour of the response from trial to trial. (This early physiological maturation of movement sensitive neurones in kitten C-laminae was also observed by Daniels *et al.*[20].) This was in contrast to many X-like cells in the A-laminae of the kitten LGN$_d$. These X-like cells would cease responding vigorously to visual targets after one to five trials. Five of the seven W-cells responded to a sinusoidally counterphased grating of low spatial frequency (0.2–0.5 cycles per degree at a contrast of 0.6 and a modulation rate of 0.3 Hz). These were the highest spatial frequencies they would respond to. All five

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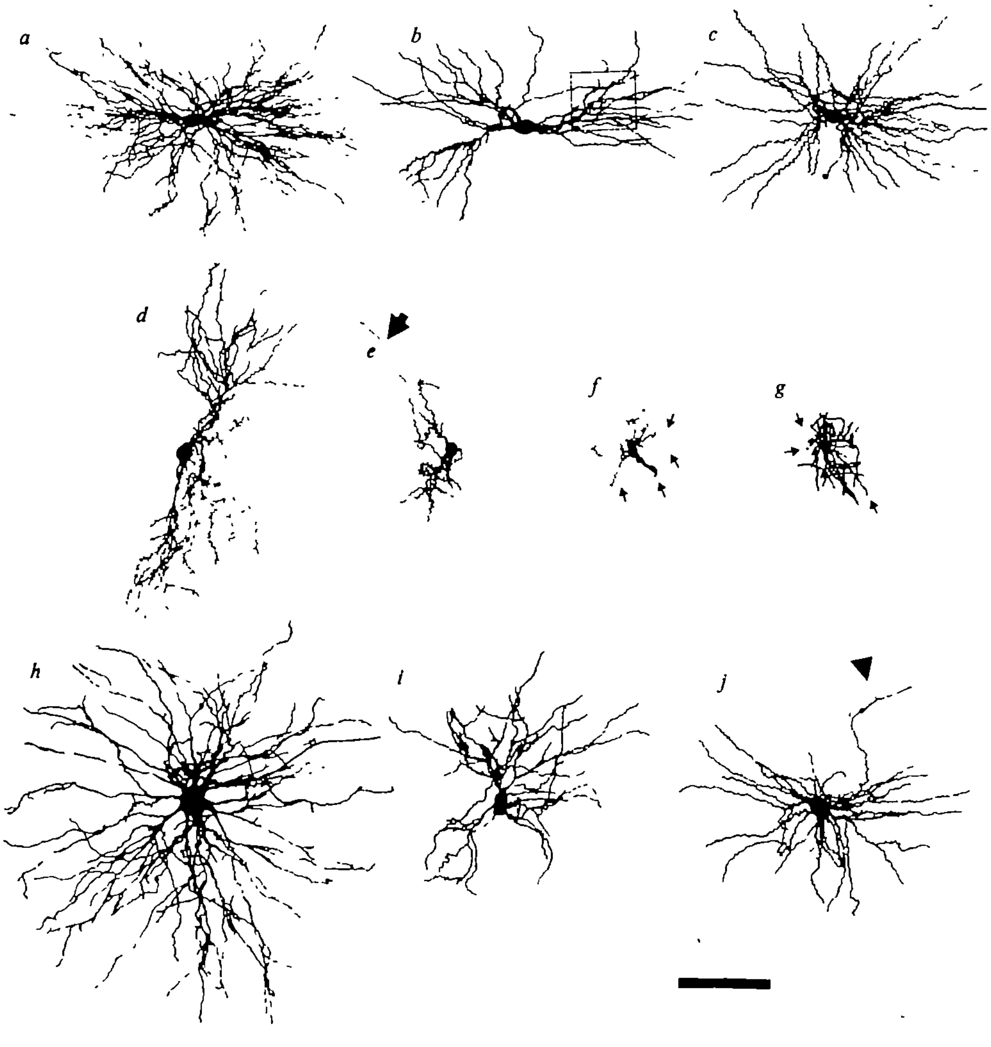

Fig. 1 Drawings of W- (*a–c*), X- (*d–g*), and Y- (*h–j*) cells from the cat dorsal lateral geniculate nucleus (LGN$_d$). *a, d* And *h* are from adult cats. *b, c, e–g*, And *i, j* are from 3–4-week-old kittens. All of the above cells were physiologically classified and then injected intracellularly with horseradish peroxidase. Drawings were made with a × 100 oil objective (numerical aperture = 1.32) and × 10 eyepieces with a drawing tube attachment. A Kodak Wratten 48A deep blue filter was used to enhance contrast when the 3–3′ diaminobenzidine reaction was used to locate the HRP filled cells. *a–c*, W-cells: note the horizontal orientation of the dendritic trees of the typical adult (*a*) and the 3–4-week old kitten (*b* and *c*) W-cells. Note the very similar appearance of the adult and kitten W-cells. *d–g*, X-cells: note the vertical orientation of the dendritic tree of the typical adult (*d*) X-cell. These X-cells in the adult also confined their dendrites to the laminae of origin. The 3–4-week old kitten X-cells (*e–g*) were morphologically immature. The arrow in *e* indicates this cell's axon. This axon had collaterals both within the laminae of origin and above the laminae in the perigeniculate nucleus (PGN). The arrows in *f* and *g* indicate terminal swellings on the ends of the dendrites of two of the very immature looking X-like cells in the kitten. *h–j*, Y-cells: the adult Y-cells (*h*) had large somata, and thick radially oriented dendrites that crossed laminar boundaries. Their axons had collaterals that innervate the PGN. The 3–4-week old kitten (*i–j*) Y-cells had a similar appearance (only smaller). They too had radially oriented dendrites that cross laminar borders and innervate the PGN (arrow in *j*). Scale bar = 100 μm for all drawings and is drawn parallel to the laminar borders of the LGN$_d$.

of these kitten W-cells showed linear summation within their receptive fields. Two of these kitten W-cells are illustrated in Fig. 1*b, c*. Note the W-cell from an adult cat's LGN$_d$ illustrated in Fig. 1*a* for comparison. The dendritic structure of the kitten W-cells is quite similar in orientation and detail to that of the adult. The dendrites are oriented parallel to the LGN$_d$ lamination. In addition to having an adult-like dendritic structure, the somata of the kitten W-cells are large (Fig. 2). The sizes of the kitten W-cells' somata overlap only with the large adult W-cells (range = 210–360 μm^2 in kitten and 80–330 μm^2 in adult). In summary, 3–4 weeks after birth, physiologically identified W-cells are structurally mature, particularly those that are sensitive to moving visual stimuli.

On the other hand, the kitten X-like cells are immature at this age. The seven X-like cells all responded to the sinusoidally modulated grating pattern in a linear fashion[4,5]. All were located in the A laminae of the LGN$_d$. In addition, they all were poorly responsive or not responsive at all to a large target of contrast opposite to their receptive field centre sign moved at moderate speeds (60–100 deg s^{-1}) through their receptive fields. Receptive field surround responses were very weak. No X-like cell responded in an excitatory fashion when the receptive field surround was presented with an annular stimulus of appropriate contrast. However, large stimuli activating both centres and surrounds simultaneously caused a reduced response in all seven X-like cells, indicating an active antagonistic surround mechanism. Latency of response to optic chiasm stimulation overlapped too much between X- and Y-cells in the 3–4-week old kittens to be a reliable correlator with other X- and Y-cell physiological properties. The structure of three of the most physiologically mature X-like cells was like that illustrated in Fig. 1*e*. These X-like cells had receptive field centre sizes ranging over 0.8–1.6°. The cell illustrated in Fig. 1*e* had many complex appendages along its dendrites which were oriented orthogonal to the lamination of the LGN$_d$ (Fig. 1*d* is an example of an adult X-cell for comparison). The other four X-like cells had morphological features that were quite different from any adult LGN$_d$ cells. Two examples of these are illustrated in Fig. 1*f, g*. Note the small dendritic arborizations and lack of obvious vertical orientation. These cells have few appendages along their dendrites. However, the dendrites terminate in swellings (arrows) which may be growth appendages. The four X-like cells with this immature structure were also the most physiologically immature (rapid decrement of a vigorous response to a visual stimulation after only one or two trials with receptive field centre sizes of 2.1–3.5°). The distribution of soma sizes for the kitten X-like cells overlaps only with the lower end of the adult X-cell size range (Fig. 2, range = 80–182 μm^2 in kitten and 80–430 μm^2 in adult). In summary, the X-like cells in the 3–4-week old kitten LGN$_d$ have some mature physiological properties (linear spatial summation and poor responsiveness to fast moving targets) and some immature physiological properties (large receptive field centres, very weak receptive field surrounds and rapid fatiguing of the cells' responses to repetitive stimulation). As a group, the X-like cells are the most anatomically immature cells in the LGN$_d$. Three of seven of these X-like cells had morphological features similar to adult X-cells except for smaller somata and dendritic spread (Fig. 1*e*), while the other four X-like cells had very short dendrites with few typical X-cell dendritic appendages. No X-cells in the

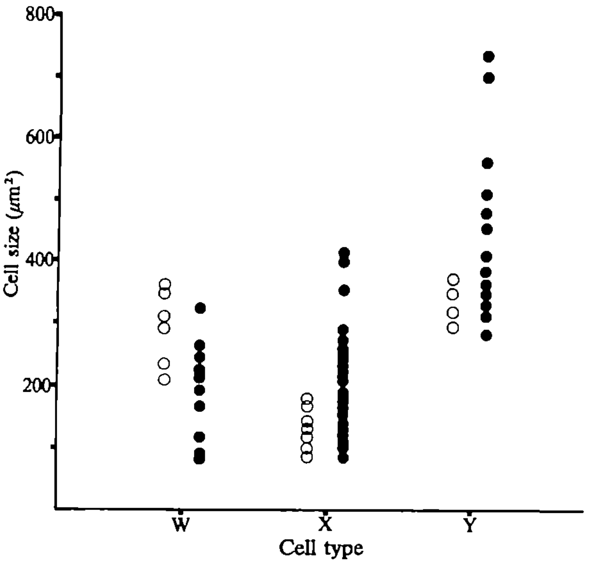

Fig. 2 Distribution of soma sizes for W-, X- and Y-cells in the 3–4-week old kitten LGN$_d$ and the adult cat LGN$_d$. Open circles, kitten cell sizes; filled circles, adult cell sizes. Adult cell sizes are taken from study of Friedlander *et al.*[10]. All cells were physiologically classified and injected intracellularly with HRP. Tissue was vibratomed, reacted with DAB, dehydrated in alcohols and cleared in xylenes in an identical fashion in the kittens and adults. Soma sizes were measured with an electronic planimeter (10 measurements averaged for each cell). Note that the kitten W-cells are as large as the largest adult W-cells, while the kitten X- and Y-cell sizes only overlap with the lower end of the adult range.

kitten or the adult had dendrites which crossed laminar boundaries in the LGN$_d$.

The four kitten Y-cells all responded in a nonlinear fashion to the sinusoidal grating modulation. They all responded regularly to a large visual target moved rapidly through the receptive fields (both of these are features of adult Y-cells[10]). Repeated presentation of stationary visual stimuli elicited consistent responses from the Y-cells. This is in contrast to the X-like cells, which fatigued readily. The Y-cells responded to appropriate stimulation of the receptive field surround in an excitatory fashion. In addition, simultaneous activation of receptive field centres and surrounds antagonized the response. These properties are similar to adult Y-cells. All of the Y-cells were located in the A-laminae as were the X-like cells. Two of these kitten Y-cells are illustrated in Fig. 1*i*, *j*. Note the adult Y-cell in Fig. 1*h* for comparison. These physiologically mature Y-cells of the kitten are similar in morphology to their counterparts in the adult. However, note that the size of the somata and area covered by the dendrites is somewhat smaller than in the typical adult Y-cell. The dendrites of all four kitten Y-cells crossed laminar borders in the LGN$_d$ as did the dendrites of 18 of 19 of the adult Y-cells. In addition, the axons of all four kitten Y-cells innervated the perigeniculate nucleus (PGN) as illustrated by the arrow in Fig. 1*j*. This type of axon collateral is quite common for adult Y-cells[10]. The somata of the kitten Y-cells that show this early physiological maturation are all in the lower end of the adult Y-cell size range (Fig. 2, range = 290–380 μm^2 in kitten and 285–750 μm^2 in adult). In summary, there is a population of Y-cells in the LGN$_d$ of 3–4-week old kittens that has mature physiological properties (nonlinear spatial summation within their receptive fields, consistent responses to repeated presentations of stationary visual targets, and responsiveness to targets moved rapidly through their receptive fields). These cells are also structurally very similar to adult Y-cells (relatively large somata, thick radially arranged dendrites, dendrites that cross laminar boundaries and innervation of the perigeniculate nucleus).

Until recently little was known about the postnatal maturation of the structure of LGN$_d$ A-laminae cells[21,22] and nothing of the development of the cells in the C-laminae. The data presented in this report support the hypothesis of differential morphological development of the functional subgroups of LGN$_d$ neurones. A population of Y-cells matures by 3–4 postnatal weeks while X-like cells are later to mature. In previous electrophysiological studies of kitten LGN$_d$ cells[20] and retinal ganglion cells[23,24] a definitive property of adult Y-cells (nonlinearity of spatial summation) was observed infrequently. This observation along with the reported greater sensitivity of the Y-system to the effects of monocular visual deprivation[12–18] during development suggests that the Y-cells are late to mature. It was therefore surprising to find that as a group the Y-cells of the kitten were more structurally mature than the X-like cells. However, other Y-cells may still mature later. At least two possible mechanisms could explain this. Some Y-cells may be so immature as not to generate action potentials and would be missed by the micropipettes. Alternatively, some of the X-like cells in the kitten may develop into Y-cells later. The neural circuitry of the retina which contributes the nonlinear subunits[5] may not be mature yet. An LGN$_d$ Y-cell that is innervated by such a retinal Y-cell would respond to a modulated grating pattern in a linear fashion and would be physiologically classified as an X-cell in the kitten. Since the results of other physiological tests used such as latency to optic chaism stimulation overlap in the kitten, such a cell can only be classified as X-like. The possibility that the LGN$_d$ Y-cell population may be further subdivided into groups that mature at different rates is further supported by studies of monocular visual deprivation[12–18]. These studies have all found that some normally responsive Y-cells develop in the LGN$_d$ laminae innervated by the visually deprived eye. However, fewer of these normal Y-cells occur in deprived laminae versus normal laminae. Other Y-cells develop abnormal structure and function after monocular visual deprivation[17]. The early maturing (structurally and functionally) Y-cells described in the present study may represent those normal Y-cells found in the adult that are resistant to the effects of visual deprivation.

The early morphological development of the W-cells in the C-laminae includes somatic growth (Fig. 2) as well as dendritic structure (Fig. 1*b*, *c*). This contrasts with the case in the A-laminae where somatic growth continues until 5–6 postnatal weeks of age[25–27]. If the soma size of the LGN$_d$ neurones relates to the size of their axonal arborization in visual cortex[28,29], the W-cells may thus have a relatively larger sphere of influence in the young kitten's cortex than X- and Y-cells. Along with the early physiological maturity of movement sensitive W-cells, this would suggest that the W-pathway may have an important role in visual processing in the young animal.

The variable rates of physiological maturation of neonatal LGN$_d$ cells are likely to depend on the development of the retinal ganglion cells that innervate them. However, the structural development of the LGN$_d$ cells may depend on intrinsic factors as well as retinal input.

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Monocular deprivation affects X- and Y-cell retinogeniculate terminations in cats

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The X- and Y-cell pathways in cats form two functionally distinct, parallel systems from the retina through the lateral geniculate nucleus (LGN) to the visual cortex[1–4]. We recently used the technique of intraaxonal injection of horseradish peroxidase (HRP) to demonstrate major differences between X- and Y-cells in their retinogeniculate termination patterns[5] (compare Figs 1*a* and 2*a*). Normally, axons of X-cells innervate geniculate lamina A or A1 (depending on the eye of origin) in narrow zones oriented perpendicular to the lamination. Some X-cells also terminate sparsely (that is, with few boutons) in the medial interlaminar nucleus (MIN), a subdivision of the LGN. Y-cell axons terminate either in laminae C and A (from the contralateral retina) or in lamina A1 (from the ipsilateral retina) in broad zones[5,6], and most also terminate densely (with many boutons) in the MIN. We now report that cats raised with monocular lid suture develop abnormal retinogeniculate termination patterns. Many X-cell axons arising from the deprived eye have unusually broad terminal fields in lamina A or A1, and some also densely innervate the MIN. Many Y-cell axons from the deprived eye have dramatically shrunken or absent terminal fields in the A laminae and MIN. These changes constitute the most peripheral effects of monocular deprivation discovered so far, are consistent with previous reports of functional abnormalities among deprived geniculate neurones[4,7,8] and suggest possible mechanisms by which the visual environment influences neuronal development.

Previously described methods[5,9,10] were used to obtain results from nine adult cats (>8 months old) raised with continuous monocular deprivation from the first postnatal week. Briefly, recording micropipettes filled with 3% HRP, 0.2 M KCl and 0.05 M Tris were bevelled to 90–120 MΩ. An optic tract axon was first recorded extracellularly and identified as X or Y by standard criteria[10,11], including axonal conduction velocity (derived from response latency to electrical stimulation of the optic chiasm) and linearity of spatial summation to visual stimuli (measured by responses to counterphased, sine-wave gratings). Each axon was then impaled, its identity as X or Y was verified intracellularly, and HRP was iontophoresed into it[5,9]. After sufficient survival time, the brain was sectioned coronally at 100 μm and reacted with 3-3′-diaminobenzidine plus cobalt chloride[12]. The retinas in four animals were dissected free, flat-mounted on slides and reacted with *o*-dianisidine[13].

We successfully injected and recovered 9 X- and 11 Y-cell axons from deprived retinas and 11 X- and 7 Y-cell axons from nondeprived retinas. Axonal morphology in lid-sutured animals was also compared with that of 16 X- and 11 Y-cell axons recovered from normal, adult cats[5]. All axons from the nondeprived eye appeared identical in their termination patterns to axons in normal cats. Among the deprived axons, however, 4 X-cells and 8 Y-cells developed abnormal morphology. Nonetheless, we found no obvious physiological differences between deprived and nondeprived axons.

Monocular deprivation increases the density (number of boutons) and extent of many X-cell terminal fields. Figure 1 illustrates two X-cell axons from the deprived eye. The terminal field in Fig. 1*a* has apparently normal morphology, being restricted to a narrow zone in lamina A1. As in other normal axons, a thin medialward branch enters the brachium of the superior colliculus but could not be traced to any terminal zone. In contrast, a representative example of a morphologically abnormal X-cell terminal arbor is shown in Fig. 1*b*. The axon exhibits an abnormally wide terminal field in lamina A1, and it also has many more terminals in the MIN than any normal

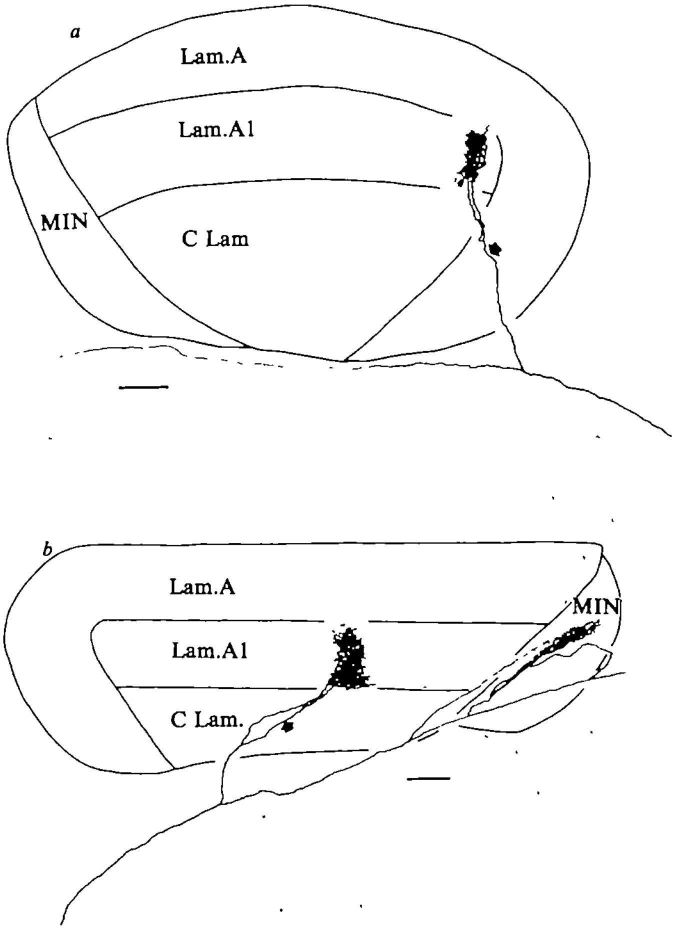

Fig. 1 Geniculate terminal zones of X-cell axons from the deprived eye Terminal zones of these X- and Y-cell axons were circularly symmetrical in the horizontal plane, and did not show obvious variations in size that could be related to eccentricity or type of centre response (that is, ON or OFF). *a*, Axon from the ipsilateral eye with apparently normal terminal pattern for an X-cell. This axon has 788 terminal boutons in lamina A1, which is within the range of 500–900 boutons for normal X-cell axons in lamina A or A1 Its receptive field was ON centre, had a centre diameter of 1.0°, and was located 44° from the vertical meridian and 23° below the zero horizontal parallel The axon's conduction latency from the optic chiasm to the recording and injection site (arrow) was 0 8 ms. The axon and its terminal field were reconstructed from 21 serial, 100-μm thick, coronal sections. Scale bar, 250 μm. *b*, Axon from the ipsilateral eye with abnormal terminal pattern. The extent of terminal field in either lamina A1 or the MIN exceeds any seen for normal or nondeprived X-cells. Thus, this axon has 1,077 terminal boutons in lamina A1 and 175 boutons in the MIN (compared with 7–31 boutons for normal X-cell axons that innervate the MIN). This axon's receptive field was OFF centre, had a centre diameter of 0 6°, and was located 11° from the vertical meridian and 3° below the horizontal zero parallel. Its conduction latency from the optic chiasm to the recording and injection site (arrow) was 0.7 ms. The axon and its terminal field were reconstructed from 17 serial, 100-μm thick, coronal sections. Scale bar, 250 μm.

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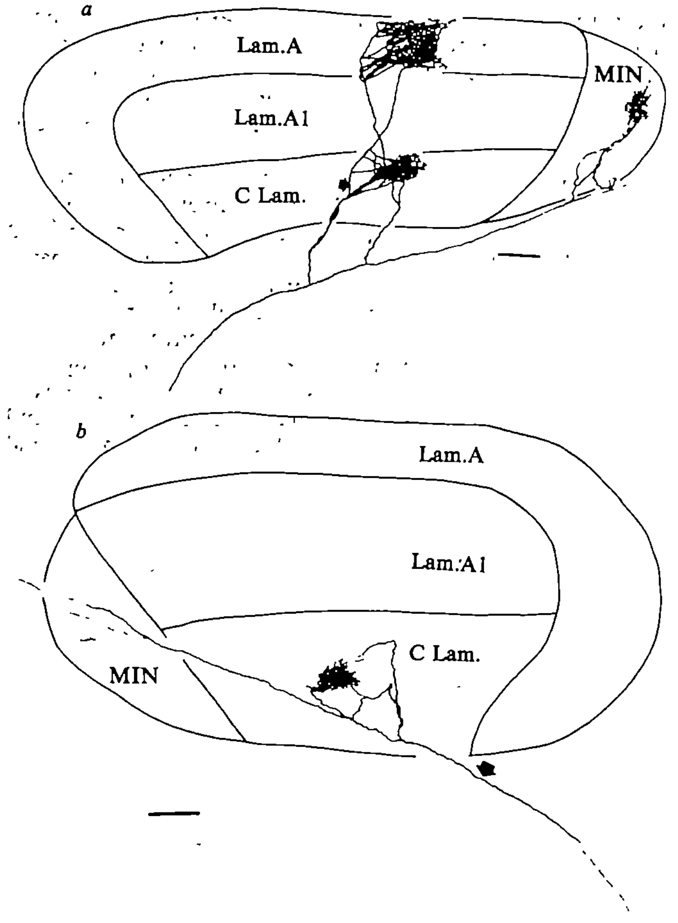

Fig. 2 Geniculate terminal zones of Y-cell axons from the deprived eye. *a*, Axon from the contralateral eye with apparently normal termination pattern for a Y-cell. This axon has 683 terminal boutons in lamina A (the range for normal Y-cell axons in lamina A or A1 is 650–1,400 boutons) and 121 terminal boutons in the MIN (normal range 90–170 boutons). Its receptive field was OFF centre, had a centre diameter of 1.3°, and was located 4° from the vertical meridian and 7° below the zero horizontal parallel. The axonal conduction latency from the optic chiasm to the recording and injection site (arrow) was 0.5 ms. The axon and its terminal field were reconstructed from 19 serial, 100-μm thick, coronal sections. Scale bar, 250 μm. *b*, Axon from the contralateral eye with abnormal terminal pattern. The axon terminates densely only in the C-laminae, has no terminal boutons in the A-laminae and sparsely innervates the MIN with only 6 boutons. This contrasts sharply with the normal pattern described in the text. This axon's receptive field was OFF centre, had a centre diameter of 1.6°, and was located 7° from the vertical meridian and 40° below the zero horizontal parallel. Its axonal conduction latency from the optic chiasm to the recording and injection site (arrow) was 0.4 ms. The axon and terminal field were reconstructed from 18 serial, 100-μm thick, coronal sections. Scale bar, 250 μm.

X-cell axon seen to date. Figure 3 summarizes some of these phenomena for arbors in the A-laminae.

Monocular deprivation affects Y-cells even more dramatically than it does X-cells, but in a different way. Figure 2*a* shows a normal Y-cell terminal field from the deprived retina. The axon terminates densely in laminae A and C, plus the MIN. In contrast, Fig. 2*b* illustrates an example of a deprived Y-cell axon that terminates densely in the C-laminae, sparsely in the MIN and not at all in the A-laminae. In normal cats, every Y-cell axon found terminates densely in lamina A or A1, and the majority that terminate in the MIN do so densely there[5,6]. Of six deprived Y-cell axons from the contralateral eye, four innervate only the C-laminae and do not terminate in lamina A. Four additional deprived Y-cell axons (one contralateral, three ipsilateral) have terminal fields in the A-laminae that are narrower and with fewer boutons than normal. Thus, deprived Y-cell terminals are most affected in the binocular segment of the A-laminae (Fig. 3), less so in the MIN, and seem normal in the C-laminae. The clearest effect of monocular lid suture on retinogeniculate Y-cell terminations, however, is that many contralateral Y-cell axons innervate only the C-laminae and do not innervate lamina A. If these four axons that fail to innervate lamina A are removed from the comparison in Fig. 3, the remaining Y-cell terminal fields are only marginally smaller than those of the normal and nondeprived Y-cells. More data are needed to determine whether the effects of deprivation are continuous such that some Y-cell terminal zones in the A-laminae are missing, some smaller than normal, and some normal, or whether the effects cause complete elimination of some Y-cell terminal fields with no effect on others.

We are confident that the abnormally small terminal fields seen in many deprived Y-cell axons are not due to incomplete or poor staining. These axons were darkly stained, as were the nondeprived and normal axons, and each axonal branch could be traced to a terminal bouton. Also, the same tissue often included axons with normal and abnormal morphology.

Figure 4 shows the retrograde labelling obtained for the somata of two identified axons that were filled with HRP. Such labelling was obtained for four X- and four Y-cells in deprived retinas and three X- and five Y-cells in nondeprived retinas. X-cell somata were consistently smaller than Y-cell somata[14], but no obvious size differences could be seen between the deprived and nondeprived somata despite large changes in the terminal field extents of the former. However, such comparisons are complicated by differences in retinal eccentricity[15,16].

Many studies have established that monocular deprivation causes abnormalities to develop in the cat's central visual pathways[4]. The expanded X-cell and reduced or absent Y-cell retinogeniculate projections described here constitute the most peripheral morphological effects of deprivation seen so far. Furthermore, although our study seems inconsistent with a recent ultrastructural study[17] that reported normal numbers and structure for retinal synapses in the deprived A-laminae,

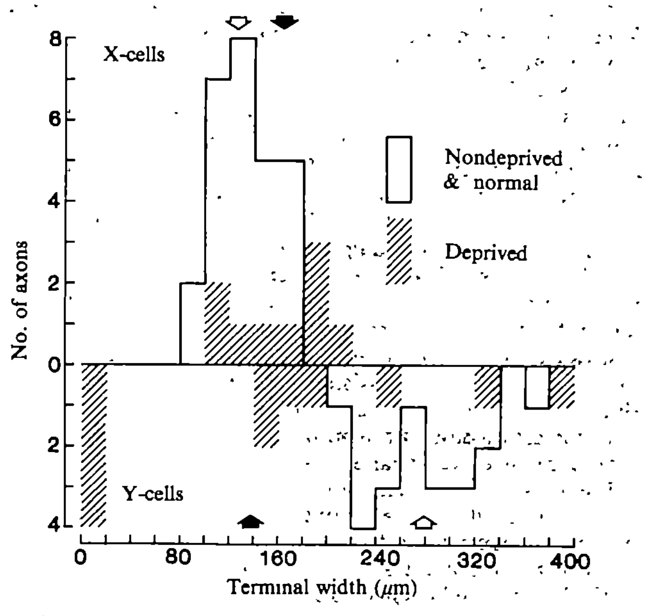

Fig. 3 Maximum widths of terminal zones in the A-laminae for X- and Y-cell axons with receptive fields in the binocular segment. The widths were measured along a plane parallel to the lamination. Because this is the plane of circular symmetry for these terminal fields and there is no effect of eccentricity or centre type on their size (see Fig. 1 legend), it seems unlikely that differences in terminal field widths result from histological artefacts. Data for nondeprived axons and axons from normally reared cats were pooled, as they are morphologically indistinguishable. Open arrows denote mean widths for normal and nondeprived axons; closed arrows, for deprived axons. Terminal zones for deprived X-cell axons are larger than those for nondeprived or normal X-cell axons ($P < 0.05$ on a Mann–Whitney *U*-test for either comparison). These zones for deprived Y-cell axons are smaller than are those for nondeprived or normal Y-cell axons ($P < 0.01$ on a Mann–Whitney *U*-test for either comparison). However, if the four contralateral Y-cell axons that do not innervate lamina A are omitted from the comparison, the difference between deprived and nondeprived or normal Y-cell terminal widths in the A-laminae is less significant ($0.05 < P < 0.1$ on a Mann–Whitney *U*-test).

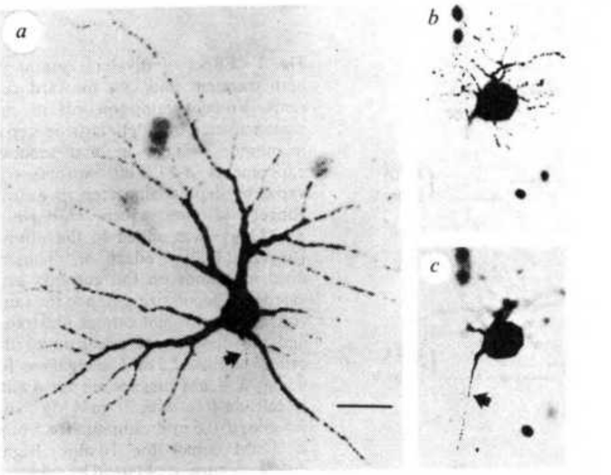

Fig. 4 Photomicrographs of retinal ganglion cells labelled retrogradely following HRP injection into their axons. Each ganglion cell was identified unambiguously by comparing its location relative to retinal blood vessels with plots of the vessels and receptive field made during the recording. *a*, Y-cell in nondeprived retina. The neurone's receptive field was OFF centre, had a centre diameter of 2.6°, and was located 17° from the vertical meridian and 45° below the zero horizontal parallel. The morphology of this Y-cell is characteristic of α cells[14-16]. The arrow points to the axon. Scale bar, 50 μm and applies as well to *b* and *c*. *b*, *c*, Two focal planes of same field of view showing an X-cell in nondeprived retina. The neurone's receptive field was OFF centre, had a centre diameter of 0.9°, and was located 20° from the vertical meridian and 5° below the zero horizontal parallel. The morphology of this X-cell is characteristic of β cells[14-16]. The focus is adjusted for the dendrites in *b* and for the soma and axon (arrow) in *c*.

the two sets of data may actually be compatible. Prior evidence suggests that retinal X-cells outnumber Y-cells by roughly 10 to 1 (refs 15, 16). Even with roughly twice as many boutons from Y-cell axons as from X-cell axons[5], retinogeniculate synapses in the A-laminae arising from X-cells probably outnumber substantially those from Y-cells. It is impossible to distinguish between X- and Y-cell retinogeniculate synapses in conventional ultrastructural material[18]. Thus, if one were to study these synapses in deprived laminae without knowledge of X- and Y-cell identity, a fairly substantial loss of terminals from the less numerous Y-cell axons with a slight expansion of terminals from more numerous X-cell axons might result in ultrastructure that appeared fairly normal. On the other hand, our data are consistent with both the loss of recorded geniculate Y-cells from deprived laminae[7] and the finding that many deprived geniculate neurones that normally accept only Y-cell input from the retina accept or retain X-cell input instead[8].

The abnormal terminal arbors we have described suggest that deprivation might cause retinogeniculate X-cell connections to expand at the expense of Y-cell connections by some sort of competitive interaction during development. X-cell axons normally seem to innervate the lateral geniculate nucleus before Y-cell axons do[19,20]. Perhaps, as in many developing pathways[21-23], the immature X-cell pathway is exuberant and is 'pruned back' in competitive interactions with the later-developing Y-cell inputs. The ability of Y-cell axons to dislodge the already-present X-cell axons may be severely reduced during visual deprivation. Consistent with this hypothesis is our observation that deprived Y-cell terminals are most severely affected in the A-laminae, where geniculate X-cells are concentrated. One means of testing this hypothesis would be to study the termination patterns of X- and Y-cell retinogeniculate axons in developing kittens.

Regardless of the developmental mechanisms, our evidence clearly shows that the visual environment can influence the formation of retinogeniculate terminations. Early visual deprivation causes abnormalities in these connections, which most seriously affect the Y-cell pathway. This might well represent the neural basis of amblyopia seen in monocularly deprived cats, as the Y-cell pathway may have a crucial role in normal vision[24].

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Action potential repolarization may involve a transient, Ca^{2+}-sensitive outward current in a vertebrate neurone

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Repolarization of the action potential in squid axon[1] and several types of neurones[2-4] involves a voltage-activated potassium (K^+) current. Voltage clamp analysis has demonstrated that this current has rapid activation kinetics[1,3-5]. In several neuronal types, the same technique has also revealed a slowly activated K^+ current that is calcium (Ca^{2+})-sensitive[3,5-10]. This slow Ca^{2+}-activated K^+ current is the major current underlying the late, slower portion of the after-hyperpolarization following an action potential[11-14]. In several muscle types, fast, transient Ca^{2+}-dependent K^+ currents have been described[15-17] which may contribute to repolarization of the action potential. Rapidly activating, Ca^{2+}-dependent K^+ currents have been observed in sympathetic neurones of the bullfrog and it has been suggested that they contribute to action potential repolarization of those neurones[8,9,18]. We have studied the membrane currents in bullfrog sympathetic neurones using voltage clamp methods and report here a transient outward current that appears to be composed of two separate currents. One of those currents is a transient, Ca^{2+}-sensitive outward current as indicated by a significant reduction of the current by treatments that reduce or block Ca^{2+} entry (Mn^{2+}, Cd^{2+}, Co^{2+}, Mg^{2+} or Ca^{2+}-free Ringer). Such treatments also decreased the rate of action potential repolarization. The results suggest that this current is involved in repolarization of the action potential and consequently may regulate Ca^{2+} entry into the neurone during spike activity.

The IXth and Xth paravertebral sympathetic ganglia of bullfrog (*Rana catesbeiana*) were excised, pinned in a Plexiglass recording chamber and continuously superfused with a Ringer's solution of the following composition (mM): NaCl 117; KCl 2.5; $CaCl_2$ 1.8; HEPES 1 (pH 7.3). Most preparations were

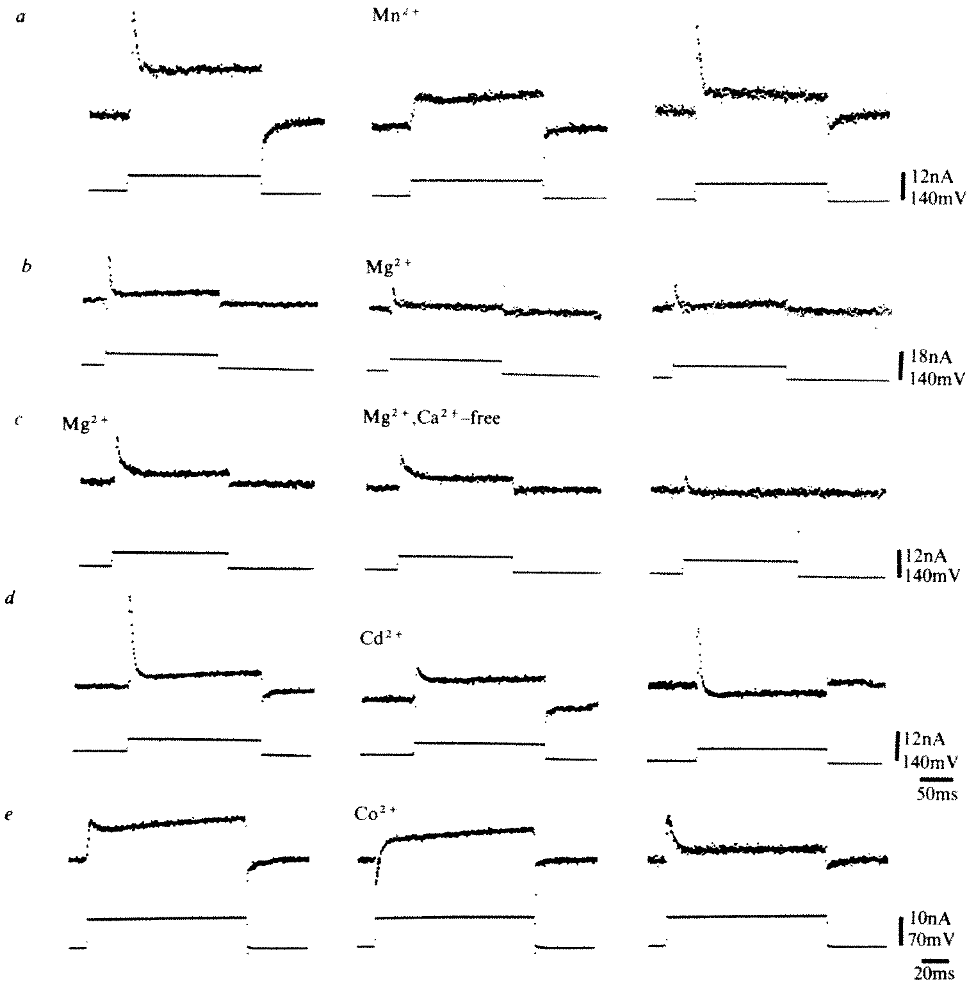

Fig. 1 Effect of divalent cations on both transient and late outward currents. Voltage-clamp records of currents activated by a depolarizing step of membrane voltage in five separate experiments (*a–e*). Left: currents activated by depolarizing step in normal Ringer's solution, except *c* in which 8 mM Mg^{2+} was added to the normal Ringer. Middle: effect of changing divalent cations on the currents activated by a depolarizing step to the same voltage; the divalent cations illustrated and the duration of exposure to the cations are: *a*, 2.5 mM manganese for 9 min; *b*, 8 mM magnesium for 4 min; *c*, calcium-free with 10 mM Mg^{2+} for 6.5 min; *d*, 0.5 mM cadmium for 5 min; *e*, 3 mM cobalt for 16 min. Right: difference current obtained by subtracting the currents recorded with altered divalent cations (middle) from control currents in normal Ringer (left). In the difference record in *d*, the inward current that follows the transient outward current resulted from an increased leak current which occurred in Cd^{2+}. An increased leak was not observed in the presence of the other divalent cations. In *a–e* the depolarizing step was to −15 mV. The holding potentials were: *a*, −92 mV; *b*, −82 mV; *c*, −78 mV; *d*, −80 mV; *e*, −85 mV. Experiments were conducted using the two-microelectrode voltage-clamp technique, as described previously[19]. The records are from data stored by a PDP 11/03 microcomputer. The current signal was filtered for frequencies above 1 or 3 kHz, which is similar to the settling time of the clamp; therefore the peak amplitude of fast current may be underestimated.

treated with 1.0% trypsin for 5–7 min followed by a 10-min exposure to trypsin inhibitor before final desheathing of the ganglion. Large neurones (>50 μm) were selected for impalement. Microelectrodes were filled with 3 M KCl and had an electrical resistance of 25–40 MΩ. Two microelectrodes were used for voltage-clamp experiments as described previously[19]; a single microelectrode was used for recording the action potential.

Both the early and late currents activated by depolarizing steps of membrane voltage and the effect of various treatments that reduce Ca^{2+} entry on those currents are illustrated in Fig. 1. The records on the left in Fig. 1 show the currents activated by depolarizing voltage steps in five different experiments (*a–e*). At the initiation of the voltage step there was a quickly activating, transient outward current that decayed rapidly. It was followed by a slow outward current that has been described previously[7–10]. The middle records illustrate the effect of 2.5 mM manganese (*a*), 8 mM magnesium (*b*), Ca^{2+}-free Ringer (*c*), 0.5 mM cadmium (*d*) and 3 mM cobalt (*e*). The transient outward current was significantly reduced by each of these treatments. The records on the right are the difference currents, which were obtained by subtracting the currents recorded in the altered divalent cation Ringer (middle) from the currents recorded in normal Ringer's solution (left). The right-hand records thus show the currents that were sensitive to either the addition of a Ca^{2+} blocking divalent cation or the omission of Ca^{2+} from the Ringer's solution.

Figure 2 shows the transient outward current recorded at a rapid sampling rate. Voltage control of the soma was achieved before the current reached peak amplitude. The development of this current with a depolarizing voltage step from −59 to −21 mV is shown in Fig. 2*a*. Following the return of membrane potential to −49 mV, a fast outward tail can be observed. In Fig. 2*b*, an inward current is seen before the transient outward current. Figure 2*c* shows two pairs of currents recorded from the same cell before and during exposure to 3 mM Co^{2+}. In normal Ringer (Fig. 2*c*i), the transient outward current developed quickly with the peak amplitude depending on the holding potential (−76 and −104 mV). In the Ringer containing Co^{2+} (Fig. 2*c*ii), an inward current was revealed followed by a more slowly developing, but early, outward current. The divalent cation-sensitive component of the transient outward current was a labile current. It seemed to be present only in cells that had resting potentials > −40 mV and spike amplitudes >80 mV following impalement with two electrodes. The amplitude of the transient outward current declined slowly with time under clamp, making recovery from a divalent cation block difficult to demonstrate. We have, however, observed recovery of both the transient and slow outward currents following exposure to Mg^{2+}, which washes out relatively quickly.

Figure 3 illustrates the effect of membrane holding potential on the activation of the transient outward current. Figure 3*a*i shows examples of the currents activated in normal Ringer's solution from membrane holding potentials from left to right of −28, −37, −55 and −92 mV. Figure 3*b* is a graph of the amplitude of the transient outward current as a function of membrane holding potential. In normal Ringer's solution, the current was activated over the range of holding potentials tested, from −105 mV to −23 mV. The amplitude of this transient current decreased as membrane holding potential was made more positive, suggesting a voltage-dependent inactivation process. The strong dependence of the transient current on holding voltage is not characteristic of the slower, 'steady-state' currents. In most cells there was a decrease in the slope of the inactivation curve at membrane holding potentials near both −60 mV and −90 mV (Fig. 3*b*). The currents activated

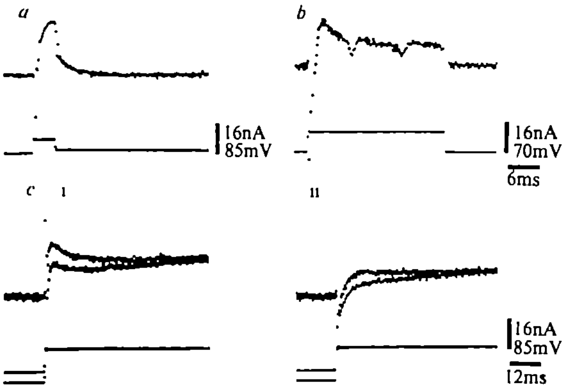

Fig. 2 Transient outward current recorded at a rapid sampling rate. *a*, Transient outward current activated by a voltage step from −59 to −21 mV. Shortly after the current peaked, membrane potential was stepped to −49 mV; this was accompanied by an outward tail current (filtered at 1 kHz). *b*, Response of a different cell clamped at −59 mV and stepped to −15 mV (filtered at 10 kHz). In this case, the transient outward current was preceded by an inward current. The two inward current events riding on the decaying outward current are presumably from axonal spikes. *c*, Current records from the same cell before (ci) and during (cii) exposure to a Ringer's solution containing 3 mM Co^{2+}. The membrane was voltage clamped at holding potentials of −76 and −104 mV and stepped to −15 mV to activate the outward current. Filtering frequency was 3 kHz.

from the same series of membrane holding potentials in the presence of 2.5 mM Mn^{2+} are shown in Fig. 3*a*ii. The amplitude of the transient current in the presence of Mn^{2+} is plotted in Fig. 3*b*. In the Mn^{2+} containing Ringer, a transient outward current was only apparent at membrane holding potentials negative to −60 mV. In addition, the shape of the inactivation curve in Mn^{2+} differed from the curve in normal Ringer, with a single, smooth rising phase between −60 and −80 mV and a distinct flattening at more negative potentials. The voltage-dependent inactivation characteristics of this transient outward current are similar to those of the A-current, first identified in molluscan neurones[20]. Similar results were obtained with Ringer's solutions containing 0.5 mM Cd^{2+}, 3.0 mM Co^{2+}, and with Ca^{2+}-free Ringer. Figure 3*a*ii also shows that in the presence of 2.5 mM Mn^{2+}, an early inward current was revealed as in Fig. 2*c*ii. This inward current accounts for the apparent reversal of the transient outward current in Fig. 3*b*. The inward current was partially blocked by 10^{-5} M tetrodotoxin (not shown) and is presumably a Na^+ current.

The effect on action potential repolarization of treatments that reduce Ca^{2+} entry are illustrated in Fig. 4. In the presence of a Ringer's solution containing 3 mM Mn^{2+}, the rate of action potential repolarization was slowed, resulting in an increased spike duration (Fig. 4*a*, upper traces). In addition, the amplitude of the after-hyperpolarization following the action potential was reduced. These changes can be seen more clearly in the electronically differentiated records (lower traces). Figure 3*b* shows the effect of a Ca^{2+}-free Ringer on the generation of the action potential. In these records, successive traces were superimposed during both the wash-in and the wash-out of the Ca^{2+}-free Ringer, showing clearly both the decreased rate of action potential repolarization and the reduction of the after-hyperpolarization. Similar effects were also observed with 0.5 mM Cd^{2+}.

The reduction of the transient outward current by a Ca^{2+}-free Ringer indicates that at least part of the current is Ca^{2+}-sensitive. The similar effect of Mn^{2+}, Cd^{2+}, Co^{2+} and Mg^{2+} suggests that these divalent cations suppress this Ca^{2+}-sensitive component of the transient current. The divalent cations used have been found to reduce or block the entry of Ca^{2+} in many cell types[21]. In bullfrog sympathetic neurones, Cd^{2+} has been shown to antagonize the Ca^{2+} current recorded in voltage-clamp experiments[10]. Moreover, in experiments using the Ca^{2+}-sensitive dye Arsenazo III to detect intracellular Ca^{2+} in these neurones[22], Mn^{2+} has been found to block the entry of Ca^{2+} resulting from a train of action potentials (S. J. Smith, A. B. MacD., and F.F.W., in preparation). The effect of membrane holding potential indicates that the Ca^{2+}-sensitive transient outward current has characteristics that differ significantly from the characteristics of the transient outward current recorded in the presence of Mn^{2+}, Cd^{2+}, Co^{2+} or Mg^{2+}, or with omission of Ca^{2+} from the Ringer's solution which suggests the presence of two separate currents. The presence of two, transient outward currents that both decay rapidly, one of which may be Ca^{2+}-dependent, has also been identified in cardiac Purkinje fibres[23].

A Ringer's solution containing 10 mM tetraethylammonium (TEA) or 5 mM Ba^{2+} blocks virtually all of the transient outward current[9]. TEA inhibits the voltage-activated or rectifier K^+ current[6,24] and can also block Ca^{2+}-sensitive K^+ currents[25–27]. Ba^{2+} has been found to block several K^+ currents in neurones including Ca^{2+}-sensitive[27,28], delayed rectifier[29,30], and M-currents[7,9,31]. Inhibition of the transient outward current in bullfrog sympathetic neurones by TEA or Ba^{2+} suggests that both the Ca^{2+}-sensitive transient outward current and the transient outward current that is not Ca^{2+}-sensitive are carried by K^+. Further evidence that they are carried by K^+ is the observation that the amplitude of the transient outward current decreased in a Ringer containing 25 mM KCl. Similar fast K^+

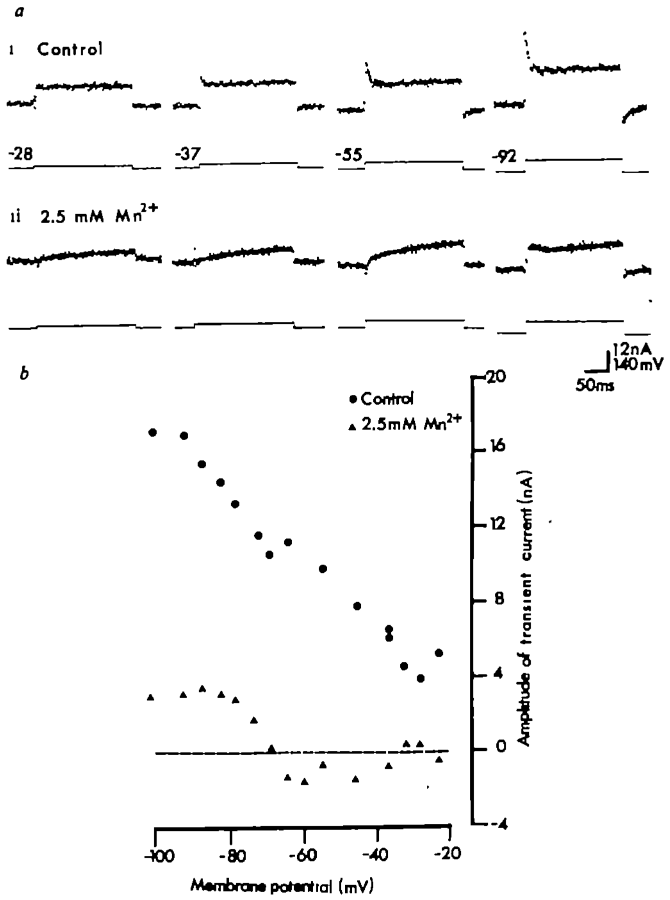

Fig. 3 Effect of membrane potential on amplitude of the transient outward current. *a*, Membrane voltage was held at a given potential for 8 s, stepped to an activation voltage of −15 mV for 200 ms, then returned to a new, more negative holding potential. (Paired pulse experiments showed that holding at a negative holding potential for more than 500 ms was necessary for recovery from inactivation.) *a*i, Effect of membrane potential on currents activated by a depolarizing step to −15 mV in normal Ringer's solution; the membrane holding voltage before each step is labelled on the voltage trace. *a*ii, Effect of 2.5 mM Mn^{2+} on outward currents activated by the same depolarizing steps from the same holding potentials in the same cell as *a*i. *b*, Graph of relationship between amplitude of the transient outward current (nA) and membrane holding potential (mV) before depolarizing step to −15 mV in normal Ringer (●) and a Ringer containing 2.5 mM Mn^{2+} (▲). Amplitude of the transient outward current was measured as the difference between the transient outward current peak and the baseline current, minus the leak current. Leak current was calculated by dividing the voltage difference of the step by the slope resistance between −65 and −90 mV. Experiments conducted using two microelectrode voltage-clamp technique[19].

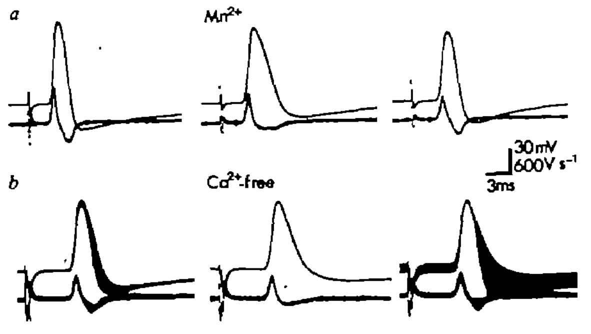

Fig. 4 Effect of divalent cations on action potential repolarization *a*, Effect of 3 mM Mn^{2+} on the action potential. Left· action potential recorded in normal Ringer's solution. Middle· action potential recorded after superfusion for 6 min with Ringer containing 3 mM Mn^{2+}. Right: action potential recorded 13 min after beginning Mn^{2+} wash-out Top trace: voltage recording of action potential. Bottom trace: electronically differentiated record of action potential. Since the addition of Mn^{2+} depolarized the cell, the membrane potential was offset to the control resting potential (−55 mV in this cell) using a bridge circuit. *b*, Effect of Ca^{2+}-free Ringer containing 6 mM Mg^{2+} on the action potential. Left: superimposed action potentials recorded initially in normal Ringer and during wash-in with Ca^{2+}-free Ringer containing 6 mM Mg^{2+}. Middle: single action potential recorded after superfusion for 10 min with Ca^{2+}-free Ringer containing 6 mM Mg^{2+}. Right. superimposed action potentials during wash-out of the Ca^{2+}-free Ringer. The resting membrane potential of this cell was −60 mV. Top trace· voltage recording of action potential. Bottom trace: electronically differentiated record of action potential. Action potentials were elicited by antidromic stimulation of the IXth or Xth spinal nerves. Records were photographed from oscilloscope trace.

currents which appear to be Ca^{2+}-sensitive or partially Ca^{2+}-sensitive have been identified in cardiac Purkinje fibres[17,32], crab muscle[16] and guinea pig myometrium[15].

The transient outward current reported here seems to be different from another fast, Ca^{2+}-activated K^+ current observed in bullfrog sympathetic neurones. Adams *et al.*[18] recently described a fast, Ca^{2+}-activated, voltage-sensitive K^+ current with an activation time constant of about 15–20 ms. The various methods used to estimate the time constant in their study involved holding the membrane potential at −10 mV. The transient outward current studied in the experiments reported here also peaks rapidly, in less than 5 ms with pulses to −10 mV; however, it inactivates rapidly following activation and thus presumably does not contribute to the currents measured in the study of Adams *et al.* As the mechanisms underlying the different properties of these two currents are unclear, the relationship between these two Ca^{2+}-sensitive outward currents is a question for future investigation.

In cat motoneurone, a fast K^+ current has been studied which is blocked by Co^{2+}, Mn^{2+}, Ni^{2+} and TEA[33]. However, these treatments did not appreciably broaden the directly activated action potential. In contrast, a decreased rate of action potential repolarization was observed in bullfrog sympathetic neurones in Ca^{2+}-free Ringer or in the presence of Ca^{2+}-blocking divalent cations. This suggests that the Ca^{2+}-sensitive transient outward current contributes to repolarization of the action potential in these vertebrate neurones. It is also possible that this current may limit the entry of Ca^{2+} into the neurone during spike activity. Since a voltage-sensitive Ca^{2+} current is activated during membrane depolarization[10,22,34], an outward current that is rapidly activated by Ca^{2+} entry would rapidly repolarize the cell thus inhibiting further Ca^{2+} entry.

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Generation of mucosal mast cells is stimulated *in vitro* by factors derived from T cells of helminth-infected rats

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The connective tissue of rats, and several other species of mammals, contains two distinct types of mast cells that differ in morphology, histochemical staining properties and location[1]. One type, frequently called the normal connective tissue mast cell, can be obtained in nearly homogeneous preparation from a mixed cell population in the peritoneal cavity and forms the basis of our knowledge of mast cells. The other type is referred to as the mucosal mast cell because in normal rats it has been observed only in mucosal tissue. Infection with helminth parasites induces an extensive accumulation of mast cells and eosinophils in the tissues[1,2], and parasites of mucous surfaces, in particular, stimulate a rapid hyperplasia of mucosal mast cells[3,4]. However, the origin of mucosal mast cells, and their relationship to the connective tissue mast cells is uncertain. We now show that lymphocytes of helminth-infected rats, on *in vitro* stimulation with specific antigen, release factors causing pronounced mucosal mastocytosis in normal rat bone marrow cultures.

Conditioned media capable of causing the proliferation of mast cells were derived from cultures of antigen-stimulated mesenteric lymph node cells of rats infected with the nematode *Nippostrongylus brasiliensis*. Figure 1 illustrates that the medium from such cultures added to normal rat bone marrow cultures encourages cell growth and enriches for mast cells. The conditioned medium also contains some growth stimulating activity for neutrophils, macrophages and eosinophils (not shown). However, restimulation and refeeding leads to cultures containing greater than 90% mast cells. By contrast, the culture

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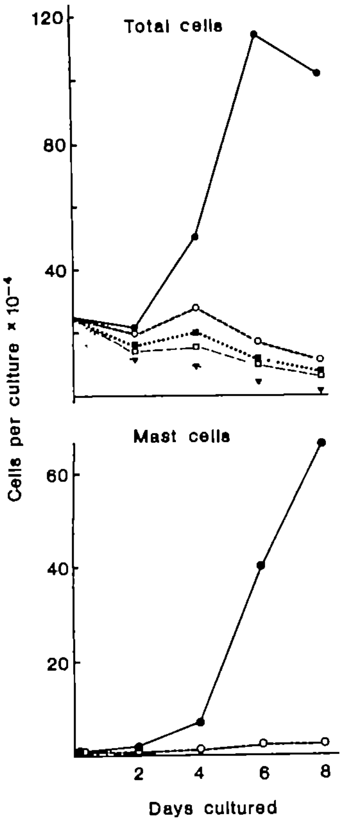

Fig. 1 Growth of bone marrow cells in the presence of culture fluids from antigen-stimulated mesenteric lymph node cells (MLN) of rats infected with *N. brasiliensis*. Shown above, in the upper section, total cells in bone marrow cultures (mean of 3) with added supernatant from: antigen-stimulated infected rat MLN (●); antigen-unstimulated infected rat MLN (○); antigen-stimulated (■) and unstimulated normal rat MLN (□); and unconditioned medium containing Nb antigen only (▼). In the lower section, mast cells in the first two of the above cultures. The bone marrow culture technique was adapted from the method of Sumner *et al.*[23]. Normal bone marrow cells from (LIS × BN) F$_1$ rats suspended at 2.5×10^5 ml^{-1} in Iscove's medium (Gibco) with 20% horse serum were cultured in 35 mm tissue culture dishes at 37 °C in a humidified incubator flushed with 5% CO$_2$ in air. Any effect of small amounts of endogenous growth stimulating activity is not detected at this low cell density: these cultures thus provide a simple system for the assay of exogenous growth stimulating activity. Twenty per cent conditioned medium, obtained by culturing syngeneic mesenteric lymph node cells from normal or infected rats in the presence of *N. brasiliensis* antigen, was added to the cultures. Rats were infected with 4,000 *N. brasiliensis* larvae[24] and mesenteric lymph nodes were removed between 12 and 30 days after infection. The lymph node cells were suspended at 4×10^6 ml^{-1} in Iscove's serum-free medium[25]. *N. brasiliensis* antigen was added at 10 μl ml^{-1}. The cells were incubated for 5 days as above and the supernatant was collected and filtered. For preparation of the antigen, adult worms were removed from the intestines of infected rats on the eighth day of infection, washed by sedimentation in 0.15 M saline and incubated in Hank's medium at 37 °C for 3 h. The supernatant containing antigenic secretions and excretions of the parasites was adjusted to a protein concentration of 1 mg ml^{-1} and filtered.

medium from antigen-stimulated normal rat mesenteric lymph node cells or from antigen-unstimulated infected or normal lymph node cells does not show these properties. The necessity to stimulate the infected rat cells with antigen to obtain conditioned medium with optimal activity indicates that primed antigen-specific cells must be activated.

Cell separation experiments indicated that the mast cell growth factor is produced by immunoglobulin-negative non-adherent cells. Thus infected rat lymph node cells depleted of plastic-adherent and immunoglobulin-bearing cells yield a culture fluid of undiminished conditioning activity whereas the supernatant fluids of cultured adherent or immunoglobulin-bearing cells have little effect. These preliminary experiments suggest, therefore, that mast cell growth factor is produced by antigen-specific T cells contained within the draining lymph nodes of *N. brasiliensis*-infected rats. This conclusion is reinforced by previous observations that the proliferation of mucosal mast cells in helminth infections is dependent on the integrity of the T lymphocyte system of the host[5–7] and can be adoptively transferred by immune T cells[8]. Although there has been controversy on this point the evidence now indicates that T cells have an inductive rather than a precursor role in influencing mucosal mast cell numbers[7].

It is often difficult to distinguish mucosal from connective tissue populations of mast cells as both possess specific receptors for IgE and dense secretory granules containing proteoglycan, histamine and basic proteins. However, mucosal mast cells require special fixation techniques for their detection in tissues and differ from connective tissue mast cells in having granules which are sparser and more variable in size and which contain less strongly sulphated glycosaminoglycans and less monoamine[9]. Additionally, each type of mast cell contains a similar yet antigenically distinct chymotrypsin-like serine protease which can be used to define the type of mast cell present in any preparation[10,11].

The mast cells generated in our cultures have the following properties. Morphologically they differ from peritoneal mast cells in being smaller and having sparser granules which show a marked variation in size (Fig. 2). Following fixation of cytocentrifuge preparations in Carnoy's fluid and staining by the astra blue (*p*H 0.3)/safranin technique, the granules of the cultured mast cells stained exclusively with astra blue whereas, by comparison, those of peritoneal mast cells stained exclusively with safranin. Preparations fixed in Carnoy's fluid and stained with toluidine blue (*p*H 0.5) were metachromatic. Uptake of IgE by surface receptors of cultured mast cells was demonstrated by overnight incubation with monoclonal rat IgE (IR162) or with serum rich in IgE from *N. brasiliensis* immune rats[12] followed by a 1-h incubation in FITC-labelled purified goat anti-rat Fcε. Finally, extracts of the cultured mast cells contained a very high level of mucosal mast cell protease (26 μg per 10^6 mast cells) (Fig. 3).

Others have observed mast cell proliferation in cultures of various tissues grown in media conditioned by antigen-stimulated[13,14] or concanavalin A-stimulated lymphocytes[15–17]. In these studies the type of mast cell was not ascertained. Ginsburg and colleagues, however, have recently identified two types of mast cells in cultures of mouse lymphoid tissue grown on an embryonic fibroblast monolayer: one type, the proliferation of which was dependent on the presence of antigen-specific T cells or their products, arose from a precursor in the lymphoid cell layer; the other type, not dependent on T-cell factors, arose from cells in the fibroblast layer[18]. Although critical discriminating tests were not performed, it was suggested that the lymphoid-derived cells were mucosal mast cells and the feeder layer-derived cells were connective tissue mast cells. As the greatest differentiation and proliferation of 'mucosal' mast cells occurred with mesenteric lymph node cells and few arose from bone marrow cells[19], it was proposed that the 'mucosal' mast cell derived from a precursor in lymphoid tissue[18].

Our present results suggest that the precursor cell for the mucosal mast cells, like the precursor for the connective tissue population[20], originates in the bone marrow. The same conclusion has recently been reached independently by Crowle[21] based on *in vivo* experiments with W/W^v mice. We propose that *in vivo* the precursor cell, after migrating from the bone marrow, would normally differentiate *in situ* in the areas where both the antigen and antigen-specific T cells are to be found—a proposition supported by ultrastructural observations of infected tissues[22]. It should now be possible to analyse the signals and the steps involved in mast cell proliferation in a system which reflects and is directly relevant to the mastocytosis occurring *in vivo*.

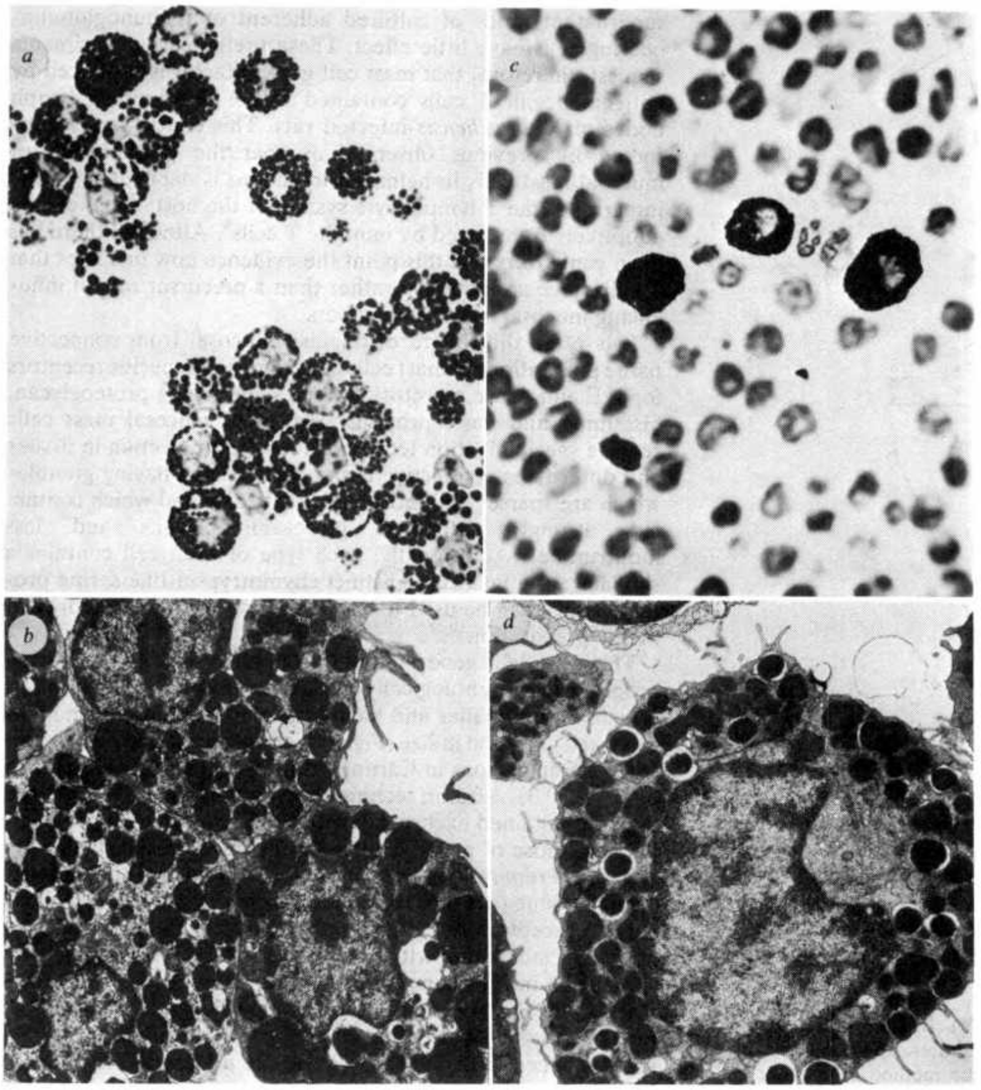

Fig. 2 Morphology of cultured mast cells. For mast cell analysis, bone marrow cells were grown in bulk cultures in 25 cm^2 tissue culture flasks. Bone marrow was allowed to grow for 2–3 weeks with weekly refeeding and restimulation with 10% fresh conditioned medium. During this time mast cells proliferated to form greater than 90% of the total cell population. Cells in suspension cultured for 21 days were fixed in Karnovsky's aldehyde fixative[26] and were pelleted and post-fixed in osmium followed by routine dehydration and Epon embedding. *a*, 1 μm section (× 745) stained with methylene blue/azure II. *b*, EM preparation (×3,800) stained in section with uranyl acetate and lead citrate. For comparison, peritoneal exudate cells from syngeneic rats treated in exactly the same way are shown: *c*, 1 μm section (×745) of peritoneal exudate cells. *d*, EM preparation (×5,300) of peritoneal mast cell.

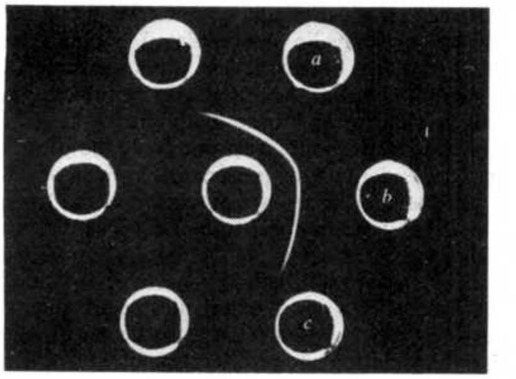

Fig. 3 Double immunodiffusion in 1% agarose to determine the presence of mucosal mast cell protease in extracts of cultured mast cells. Mucosal mast cell protease was purified from small intestine as previously described[27]. Specific antiserum against mucosal mast cell protease was prepared by injecting rabbits with 50 μg of protease with complete Freund's adjuvant at weekly intervals. Each well contained 30 μl of sample. Centre well, anti-mucosal mast cell protease; *a*, purified protease (150 μg ml^{-1}); *b*, 0.15 M KCl extract (200 μl) of pelleted cells from a culture containing 2×10^6 mast cells; *c*, 0.15 M KCl extract of syngeneic rat peritoneal cells containing 1.9×10^6 mast cells. The results show that the extract of the cultured cells contains a high level of mucosal mast cell protease indicating the presence of mucosal mast cells in the culture. The quantity of mucosal mast cell protease in the cell extract, measured by radial immunodiffusion, was 26 μg per 10^6 mast cells, representing approximately 13% of the soluble mast cell protein.

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The role of position in determining homoeotic gene function in *Drosophila*

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Homoeotic mutations of *Drosophila* lead to the replacement of one structure by another, for example, *Antennapedia*[1] replaces the antenna with a mesothoracic leg and *bithorax* produces an anterior wing instead of the anterior haltere. The transformed structures differentiated by the homoeotic mutants are essentially normal—only the position in which they appear is abnormal. The mutant phenotypes suggest that in normal development homoeotic genes are involved in developmental alternatives and contribute to a genetic address that defines the particular developmental pathway taken by a primordial group of cells[2–5]. Thus, in the absence of homoeotic gene function, primordia in different positions should follow the same basic developmental pathway. This is indeed the case for embryos that show no activity of *bithorax* genes; thoracic and abdominal segments develop as mesothorax[6]. The simplest view on homoeotic gene function is that the genes act selectively on primordia depending on their position in the embryo. To test this hypothesis, we used a mutation at the *Antennapedia* locus, *Antp*^{73b}, which transforms the antenna into a mesothoracic leg, and we observed the function of the homoeotic genes *engrailed* and *Ultrabithorax* in two apparently morphologically identical appendages which develop from primordia in different positions. Our results indicate that position is the relevant factor in the function of these two homoeotic genes.

We have considered the antennal primordium which normally gives rise to the eye, head and antenna of the fly, and the ventral mesothoracic primordium which produces the second leg. Two differences have been described for these two primordia with regard to the function of *engrailed* and *Ultrabithorax*: (1) the subdivision of the original primordium into anterior and posterior polyclones occurs at about 72 h of development (mid-larval period[7,8]) in the antennal cells, whereas it occurs at about 3 h (blastoderm period) in the mesothoracic leg[9]. As the function of the *engrailed* gene is required for maintenance of the antero-posterior boundary[10–12], this indicates that *engrailed* is active in the mesothorax much earlier than in the antennal segment. (2) Mutations at the *Ultrabithorax* locus (*Ubx*) produce an homoeotic transformation of the posterior mesothoracic leg compartment into the homologous prothoracic one[13,14]. These mutations have no effect on antennal development, which indicates that the *Ultrabithorax* gene is active in the leg but not in the antenna.

There are two sets of legs of mesothoracic type in flies heterozygous for the dominant mutation *Antp*^{73b}: one in the normal position of the second leg (henceforth referred to as the thoracic leg) and the other in the position of the antenna (to be referred to as the cephalic leg). The bristle pattern of the cephalic leg is typically mesothoracic (Fig. 1). We have studied the period of function of *engrailed* by timing the establishment of the antero-posterior boundary in the cephalic and thoracic legs and the respective requirements for *Ultrabithorax* activity.

In these experiments we induced clones that were marked genetically (for details see Table 1 legend) in different developmental stages to determine the time at which the anterior (A) and posterior (P) compartments of the leg are established. Before this time, individual clones can extend to both compartments but thereafter they cannot (see Table 1). In the thoracic leg, A and P polyclones are segregated after 4±2 h (blastoderm period) which agrees with the results obtained in non-*Antennapedia* flies[9]. By contrast, clones in the cephalic leg can extend to both compartments until the mid-larval period (84 ±12h after oviposition, *Minute* time). This is about the time when the antero-posterior subdivision occurs in the normal antenna[8].

We compared the effects of the lack of *Ubx* activity in the cephalic and thoracic legs of *Antennapedia* flies. It has been found recently[13,14] that the loss of *Ubx*$^+$ function in early embryogenesis (blastoderm stage) means that the posterior compartments of mesothoracic and metathoracic legs develop as the posterior compartment of the prothoracic one. The loss of *Ubx*$^+$ function at later times does not affect the mesothoracic leg; the metathoracic leg, however, is then transformed into a mesothoracic one. Thus, there is an early function of the *Ubx* gene necessary for the normal development of meso- and metathoracic legs and also a later function necessary for metathoracic development.

We have generated, by X-ray-induced mitotic recombination, *Ubx*$^-$ clones in *Antennapedia* flies. Genetic mosaics must be used as *Ubx* mutants are zygotic lethal. Most of the *Ubx*$^-$ clones were induced at the blastoderm stage although some were also induced during the larval period as it was conceivable that the cephalic leg might show a temporal succession of mutant phenotypes as in the case of the metathoracic leg[13]. Appropriate genetic combinations were constructed (details are given in

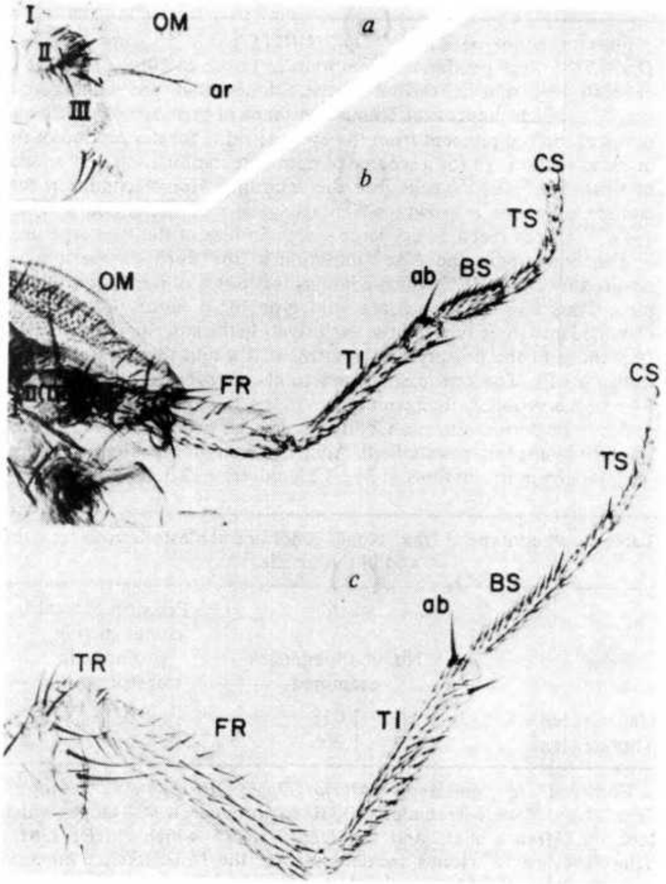

Fig. 1 The cephalic (*b*) and the second (*c*) legs of *Antp*^{73b} flies showing the close similarity between them. A normal antenna (*a*) is included for comparison. The three appendages are shown at the same magnification. The three antennal segments (I, II and III) and arista (ar) are indicated. The apical bristle (ab), typical of the mesothoracic pattern is present in both legs whereas landmarks of first and third leg patterns, such as anterior and posterior transverse rows, are absent. In general, the first antennal segment remains untransformed and the second is modified towards the trochanter. The transformation into leg of mesothoracic type is usually complete distal from the trochanter, although sometimes arista structures remain in the tarsal region. TR, trochanter; FR, femur; TI, tibia; BS, basitarsus; TS, tarsal segments; CS, claws; OM, ommatidia.

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Table 2 legend) to allow the detection of *Ubx*$^-$ clones. We found that in the thoracic (second) leg, most *Ubx*$^-$ clones induced at blastoderm showed the prothoracic transformation in the posterior compartment (Table 2). However, clones of the same genotype and initiated at the same time, failed to show prothoracic transformation in the cephalic leg and instead formed a mesothoracic pattern. In both legs, clones produced during the larval period remained mesothoracic. Thus we conclude that the thoracic leg requires *Ubx*$^+$ activity in early development but the cephalic one does not.

The results presented here demonstrate the importance of position in the function of homoeotic genes. In *Antennapedia* flies the antennal primordium, although it develops into a mesothoracic leg, retains the pattern of homoeotic gene function (at least for *en* and *Ubx*) characteristic of the normal antenna. The thoracic legs in *Antennapedia* flies develop normally with respect to *en* and *Ubx*. The critical factor for the activity of these genes seems to be the position occupied by the primordial cells, not the identity of the structure into which they develop. This conclusion, however, is based on the assumption that there is no difference other than position between the cephalic and thoracic primordia of *Antennapedia* flies. This assumption—although very reasonable, as both primordia develop into the same structure—cannot be rigorously proven at present.

Table 1 Establishment of the antero-posterior segregation in cephalic and thoracic legs

Age at clone initiation	No. of clones found in anterior and posterior compartments: Cephalic leg *n*	A	P	A+P	Thoracic leg *n*	A	P	A+P
Blastoderm (4±2 h)	1,202	12	9	15	1,330	26	10	0
36±12 h 60±12 h	832	17	14	10	—	—	—	—
84±12 h	394	17	9	1	—	—	—	—

Flies of genotype *M(1)o*^{Sp}*Dp(3;1)P115/y w f*^{36a}; *Antp*^{73b} *Ubx*^{1}/*Dp(3; 3)P5* were irradiated as embryos or larvae at 500 or 1,000 R to generate, by mitotic recombination, clones that are homozygous *y w f*^{36a} (which changes the colour and shape of bristles and cuticle and removes the red pigment from the eye; see ref. 1 for description of the markers and ref. 13 for a scheme of mitotic recombination very similar to this). The *y w f*^{36a} cells lose the retarding *Minute* condition that confers on them a growth advantage over their neighbours. Thus, *y w f*^{36a} clones reach a very large size[15]. In flies of the genotype used in this experiment the *Ubx*^{1} mutation in the third chromosome is covered by *Dp(3; 3)P5* which contains two doses of the *bithorax* complex. Thus *y w f*^{36a} clones are wild type for *bithorax*. Clones were classified into three types: those exclusively in the anterior compartment (A), those in the posterior compartment (P), and those extending to both (A+P). The one case of apparent anteroposterior crossing at 84±12 h is probably the result of two independent clones, one anterior and one posterior (the probability of such an event was 0.45 for the number of appendages studied). Age is given in hours after egg laying. The data from irradiations at 36±12 h and 60±12 h are pooled.

Table 2 Phenotype of *Ubx*$^-$ clones generated at blastoderm in cephalic and in thoracic legs

	No. of appendages examined	Fraction of total clones showing prothoracic transformation
Cephalic leg	1,018	0/18
Thoracic leg	1,008	6/9

Embryos of genotype *M(1)o*^{Sp}*Dp(3; 1)P115/y w f*^{36a}; *Antp*^{73b} *Ubx*^{1}/*Ubx*^{130} were irradiated (500 R) to produce *y w f*^{36a} clones which lack the *Minute* allele and the *Dp(3; 1)P115* which carries *Ubx*$^+$. Therefore, *y w f*^{36} clones are defective for the *Ultrabithorax* function and because of the *Minute* effect[15] reach a very large size which permits an unequivocal identification of the bristle pattern as prothoracic or mesothoracic. Clones producing prothoracic transformation are represented as a fraction of the total number of clones found in the posterior cephalic and second thoracic legs. We also found *y w f*^{36a} clones in the third posterior thoracic leg (metathoracic) which showed (8/10) prothoracic transformation. The reason why not all clones in the thoracic legs showed prothoracic transformation is purely technical. In the recombination system used there are always several *y w f*^{36a} clones which arise after mitotic recombination distal to *Dp(3; 3)P115* and hence retain the *Ubx*$^+$ gene. These have been estimated to represent 20% of the total *y w f*^{36a} clones[13,14]. Clones generated after the blastoderm period do not show prothoracic transformation in either the cephalic (0/34) or thoracic (0/10) legs, in agreement with previous results[13,14].

The lack of activity of *Ubx* in the cephalic leg poses an intriguing question about the development of the legs; if the normal development of a leg of mesothoracic type requires (at least in the posterior compartment) a decision prothorax–mesothorax[13] which is implemented genetically through one of the functions—postprothorax—controlled by the *Ultrabithorax* gene, then one would expect any leg of mesothoracic type to undergo the same decision. But in the case of the cephalic leg of *Antennapedia* that gene function is apparently not required, suggesting that either cephalic and thoracic leg primordia in *Antennapedia* produce the same structure via different sets of decisions or the postprothorax function is provided in the head by another developmentally homologous gene that is inactive in the thorax.

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Epidermal growth factor rapidly stimulates prolactin gene transcription

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Epidermal growth factor (EGF) was originally characterized as a growth factor for various cell types[1,2] and was subsequently shown to affect a number of cellular and molecular processes[3,4], of which many might be considered as a part of the pleiotropic growth response (enhanced uptake of glucose, uridine and amino acids and stimulated synthesis of protein, RNA and DNA). Very early responses to EGF, such as increased sodium fluxes[5] and stimulation of tyrosine phosphorylation[6], have been proposed to mediate some or all of EGF's effects. In a number of tissues, EGF has been shown to increase the synthesis of specific proteins[7–10]. We have investigated the effects of EGF on prolactin synthesis in the GH_4 rat pituitary cell line to gain further insight into the mechanism of EGF's actions on cellular

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functions. Addition of EGF to GH_4 cells results in a three- to sixfold stimulation of prolactin synthesis, as well as a partial inhibition of cell growth[7,8]. In this report, we demonstrate that the increased prolactin synthesis appears to be the result of a rapid stimulation of prolactin gene transcription by EGF. It is tempting to speculate that very early transcriptional effects on specific genes, such as reported here in the case of the prolactin gene in GH_4 cells, may mediate some or all of the later effects of EGF on cell cycle regulation in those cells for which it serves as a growth factor.

The synthesis of prolactin in GH_4 cells is regulated by a wide variety of peptide and steroid hormones[11-13]. Thyrotropin releasing hormone (TRH) stimulates prolactin synthesis in GH_4 cells by acting at the nuclear level to increase the production of prolactin mRNA[14]. The increased steady state content of the primary transcript of the prolactin gene suggested that a transcriptional mechanism was involved. In order to determine whether EGF was also acting at a transcriptional level to increase prolactin mRNA, total cellular poly(A) RNA from control and EGF treated cells was analysed using RNA blotting procedures after size fractionation on denaturing agarose gels[15].

Figure 1*a* shows that EGF promotes an increase in the steady state level of several nuclear prolactin RNA species including the primary transcript. Visualization of the nuclear species is enhanced by hybridizing the blotted RNA with both labelled cloned prolactin gene-specific intervening sequence and prolactin cDNA. Figure 1*b* shows a densitometric scan of the larger prolactin nuclear transcripts. In this experiment, there is approximately a fourfold stimulation of the steady state level of each of the prolactin putative nuclear precursors, including the primary transcript. This is comparable to the three- to sixfold EGF stimulation of mature prolactin mRNA levels which are typically observed.

Because of their presumed very short half life, the steady-state level of prolactin nuclear transcripts has been proposed to accurately reflect prolactin gene transcription[14]. To verify this, the time course of EGF stimulation of prolactin gene transcription was measured by elongating nascent prolactin RNA transcripts in isolated nuclei as well as by quantitating nuclear precursors by Northern gel analysis. The time course of EGF stimulation of prolactin gene transcription, as well as the resultant cytoplasmic prolactin mRNA accumulation is shown in Fig. 2. The close agreement between the two measurements of transcription confirms previous predictions that changes in nuclear RNA precursor levels can represent an accurate estimation of the transcriptional activity of specific genes.

EGF maximally stimulates prolactin gene transcription 10-fold within 1 h, from 2 to 18 p.p.m. per kilobase, using a 0.9 kilobase prolactin genomic intervening sequence probe. This stimulation is followed by a rapid attenuation, such that 3 h following EGF addition prolactin gene transcription is only half of its maximally induced level. The net effect of this burst of prolactin transcription is to rapidly elevate the cytoplasmic prolactin mRNA content. There is a further slower transcriptional attenuation occurring over a period of days and during this time the prolactin mRNA levels remain fairly constant, paralleling the slow decline in transcription. In some cases the attenuation event has been more pronounced such that transcription returns to control levels within the first 36 h, while in others it remains moderately elevated for days. Neither the initial rapid nor subsequent slower attenuation can be accounted for by reciprocal changes in total polymerase II transcriptional activity.

The data summarized in Fig. 2 demonstrate that the kinetics of prolactin mRNA accumulation in response to EGF reflect the kinetics of effects on prolactin gene transcription. Since a steady-state transcriptional effect is not achieved, the kinetics of EGF-stimulated prolactin mRNA accumulation is not a reflection of its cytoplasmic half life. Thus, the accumulation of prolactin mRNA ceases 10 h after the addition of EGF because the increased rate of production of newly transcribed prolactin mRNA sequences no longer exceeds the rate of cytoplasmic decay. When prolactin mRNA stability is estimated under conditions of steady-state transcription, the half-life is approximately 6 h (ref. 16). Following prolonged incubations with EGF, there is an increase in cytoplasmic prolactin mRNA without a concomitant increase in transcription (Fig. 2). The mechanism of this late effect of EGF is the subject of current investigation.

The mechanism of the EGF induced transcription is unclear. There are a variety of second messenger systems which have been associated with EGF. A large body of evidence suggests that the EGF receptor is a tyrosine kinase which is capable of autophosphorylation in addition to the phosphorylation of other membrane proteins[6,17,18]. Various cytoplasmic and cytoskeletal proteins may also be substrates, either directly or indirectly[19]. However, as yet, no nuclear protein has been shown to be modified by tyrosine phosphorylation in response to EGF treatment. EGF stimulation of sodium fluxes has also been suggested as a critical intermediate in the regulation of cellular processes by EGF[5]. Increased cytoplasmic sodium can affect the homeostasis of other ions including calcium[20], which has been suggested to play a role in regulating prolactin gene expression[21].

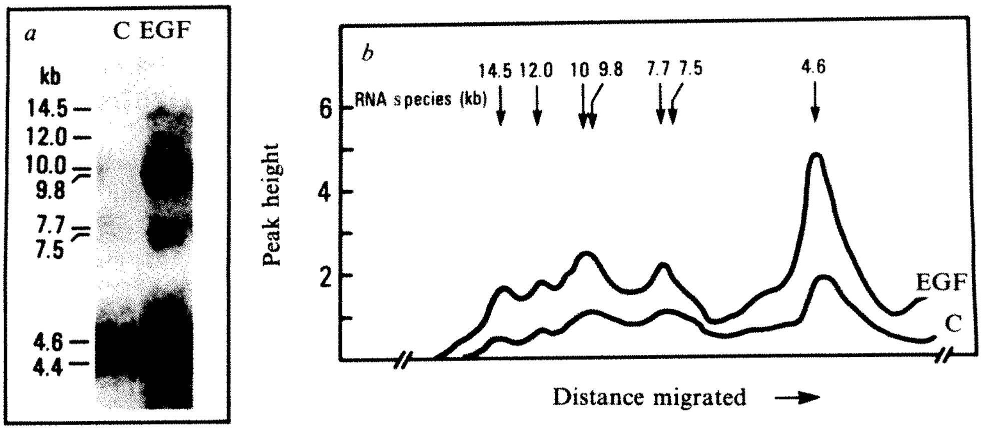

Fig. 1 Effects of EGF on the level of nuclear prolactin RNAs. *a*, Poly(A) RNA was prepared from control GH_4 cells (C) and from cells treated with 5×10^{8} M EGF for 24 h (EGF) as previously described[14]. Aliquots of 12 μg of each RNA were denatured by heating (65 °C) in sample buffer (10 mM NaH_2PO_4, *p*H 7.4, 2.2 M formaldehyde, 50% (v/v) formamide). After electrophoresis on a 1.5% agarose gel containing 1.1 M formaldehyde, the RNA was transferred to 'diazotysed' paper (DBM). The RNA was hybridized with 10^7 c.p.m. per ml of both nick-translated prolactin cDNA and prolactin gene specific intervening sequence, pIVSc (specific activity 3–8×10^{8} c.p.m. per μg with [α-^{32}P]dCTP) for 16 h at 42 °C. The autoradiogram was exposed for 2 days to clearly visualize the larger nuclear prolactin RNAs. The lower portion of the gel containing mature prolactin mRNA was drastically overexposed and is not shown. The sizes of the prolactin nuclear RNAs are extrapolated from the migration of 18S and 28S RNA. The largest (14.5 kb) species (alternatively determined to be 12.5 kb when DNA standards are used, data not shown) appears to be the true size of the gene (S. Supowit *et al.*, in preparation) and is therefore referred to as the primary transcript. *b*, Densitometric scan of the autoradiograph shown in *a*. Stimulation of each nuclear prolactin RNA (based upon comparison of peak areas) is approximately 3.8-fold.

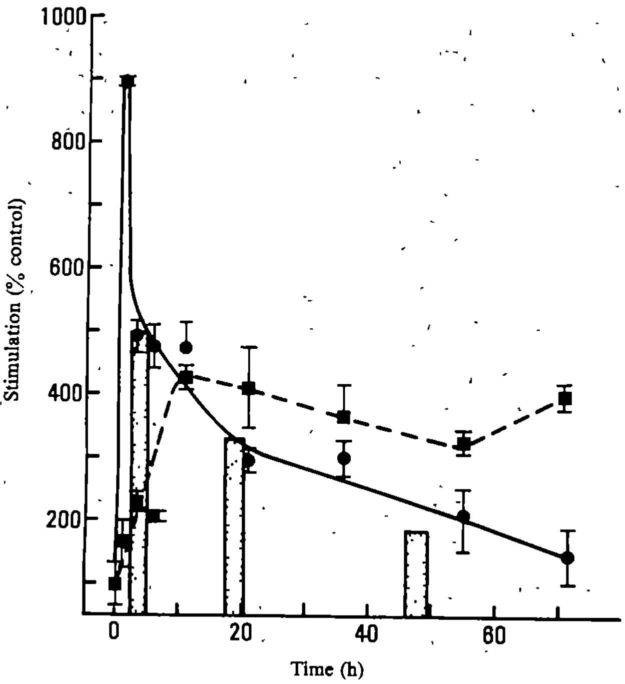

Fig. 2 Time course of EGF stimulation of prolactin gene transcription rate (●), prolactin precursor RNA level (stippled bars) and cytoplasmic prolactin mRNA (■). GH_4 cells were incubated with 5×10^{-8} M EGF for the indicated times before being collected by scraping into iced phosphate-buffered saline (PBS). After washing in PBS, cells were lysed by gentle homogenization in 150 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 10 mM Tris *p*H 7.8, 0.1% Nonidet P-40. After low-speed centrifugation, total RNA was prepared from the cytoplasm by phenol/chloroform extraction. Aliquots of 20 μg were bound to 1 cm squares of activated DBM paper and hybridized to excess nick-translated cloned prolactin cDNA to quantify prolactin mRNA content. Each point represents the mean ± s.e. of triplicate determinations. The nuclear pellets were washed in the nascent chain labelling buffer (150 mM KCl, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 10 mM Tris, *p*H 7 8, 10% glycerol). Nuclei were then incubated (25 °C, 45 min) in the same buffer supplemented with 1 mM ATP, CTP and GTP and 200 μCi [^{32}P]UTP (200–400 Ci mM^{-1}) allowing nascent transcripts to elongate. Labelled RNA was purified and quantified as described in refs 24 and 25. Briefly, $8–15\times10^6$ c.p.m. of ^{32}P-labelled RNA was hybridized to 2 μg of a 0.9 kb subcloned fragment of a prolactin gene specific intervening sequence (pIVSc) bound to a 3 mm disk of nitrocellulose Each point is the mean ± s.e. of the transcription rate (specific c.p.m. bound/c.p.m. added) based upon triplicate hybridizations. Both control and EGF stimulated prolactin gene transcription is undetectable when α-amanitin (0.8 μg ml^{-1}) is included in the incubation. The hybridization is linear with added labelled RNA and constant with variable DNA/filter (0.5–5.0 μg). The use of a cloned intervening sequence fragment during the hybridization prevents variable competition by accumulated mRNA. Total cellular poly(A) containing RNA was prepared from cells treated in parallel. Prolactin RNA precursors were quantified as described in Fig. 1. For comparison purposes all data are expressed as percent control. Basal-prolactin gene transcription rate in GH_4 cells is 2 p.p.m.; maximal stimulation is 18 p.p.m. Basal prolactin mRNA level is 1,200 molecules per cell; maximal stimulation is 5,300 molecules per cell

Alternatively, it is possible that a direct interaction of rapidly internalized EGF–receptor complexes with the nucleus mediates the transcriptional effects. However, the shape of the time course of prolactin transcriptional stimulation, a burst followed by rapid attenuation, is consistent with the time course of the binding of EGF to surface receptors in cultured cells, a transient maximum followed by rapid internalization[22]. This time course is distinct from the gradual constant accumulation of nuclear membrane bound EGF[23]. These data suggest that the putative nuclear binding events are not involved with the transcriptional regulation of prolactin.

An increase in prolactin gene transcription rate appears to be the major, if not sole, mechanism for the EGF stimulation of prolactin synthesis in GH_4 cells. The burst-attenuation kinetics of the transcriptional stimulation result in the rapid elevation of the cytoplasmic prolactin mRNA to the maximal levels. This result demonstrates that caution should be exercised in interpreting half times of accumulation as a reflection of message stability. We suggest that burst-attenuation transcriptional kinetics could be an important physiological mechanism for rapidly increasing the cytoplasmic content of specific long-lived mRNAs to new steady-state levels in response to external stimuli. The stimulation of prolactin transcription by EGF is too rapid to be a secondary consequence of any effect on the cell cycle and kinetic analysis suggests that the transcriptional effects are closely linked to the interaction of EGF with its receptor on the surface of the cell. It is likely, therefore, that regulation of prolactin gene transcription is mediated by a process that is common to the actions of EGF in other target tissues including those for which it is a positive growth factor. Transcriptional regulation of a specific protein(s) is, therefore, proposed to be among the 'early' events produced by growth factors, and may mediate the observed regulation of the cell cycle by growth factors.

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Kinetics of sickle haemoglobin polymerization in single red cells

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Kinetic studies on solutions of purified haemoglobin S indicate that the rate of intracellular polymerization is an important variable in the pathophysiology of sickle cell disease[1–4]. Until now, however, no experimental technique has been available to measure directly the kinetics of intracellular polymerization. Indirect methods, which use visual determination of cellular shape changes or changes in filterability of red cell suspensions, have given apparently conflicting results[5–9]. Here we report our initial results on the application of a laser-photolysis, light scattering technique to measure directly the kinetics of haemo-

globin S polymerization in single red cells. In our experiment, deoxyhaemoglobin S is rapidly formed by photolysing the carbon monoxide complex with an argon ion laser focused inside the cell, and the change in scattered light is used to detect the appearance of polymer. We find a very wide distribution of delay times, ranging from 1 ms to >100 s, indicating that the polymerization inside red cells proceeds by the same nucleation and growth mechanism as in solutions of purified haemoglobin S[10].

Red cells from patients with homozygous sickle cell disease were washed and diluted to a volume fraction of about 0.001 in a buffer consisting of 0.12 M sodium chloride, 2.4 mM potassium chloride, 15 mM disodium hydrogen phosphate, 2.5 mM sodium dihydrogen phosphate, 5.5 mM dextrose and $1\ \mathrm{g\ l^{-1}}$ bovine serum albumin. The oxygen in the cell suspension was exchanged for carbon monoxide, and sodium dithionite was then added anaerobically to a final concentration of 10 mM to remove any remaining traces of oxygen. The final room temperature *p*H was 7.4, and the osmolality determined from the freezing point depression was 290–300 mOsmol. Cells were sedimented onto the top coverslip of a thermostated Dvorak–Stottler flow cell[11]. An argon ion laser at 514 nm was focused inside a single red cell to a diameter of 3 μm (full width at half peak intensity) with a thermostated 90× oil immersion objective having a numerical aperture of 1.32. The laser was directed to the objective via a half-silvered mirror mounted above it at 45°. Light reflected and scattered by the red cell was collected by the objective, focused with an eyepiece onto a pinhole with a diameter of the magnified image of the cell, and projected onto a photomultiplier tube with a second eyepiece, using a Leitz MPS photometer attachment. The directly reflected laser beam was blocked by masking the centre of the tube lens mounted above the half-silvered mirror. Laser heating of the red cell was determined in experiments on cresol red to be less than 1 °C at the centre of the beam using a peak power density of $\sim 1{,}000\ \mathrm{W\ cm^{-2}}$ to achieve full photolysis.

To investigate the kinetics of intracellular polymerization at 37 °C systematically, progress curves were recorded for 453 different cells from four patients with homozygous sickle cell (SS) disease. Cells were never kept at 37 °C for more than 3 h, as previous experiments had indicated that changes in the kinetics occurred after this time. Cells were chosen at random and no results were excluded from the data analysis.

Figure 1 shows representative progress curves for sickle cells, for normal cells and for solutions of purified haemoglobin S. Normal cells gave a noisy signal which did not change with time. A variety of progress curves were observed for sickle cells. These could be roughly divided into two types. The first type resulted from the most rapid intracellular polymerization, and was very similar to that found for polymerization from solution. There was a clear delay followed by a sudden increase in light scattering, where the average increase in the scattered intensity was the same as that found for solution polymerization. A significant difference between the solution and cell progress curves was that the increase in light scattering was accompanied by a marked decrease in the amplitude of the noise (Fig. 1). The amplitude of the noise during the delay period was much greater than that found for solution, and was very similar to the noise observed for normal cells (Fig. 1). The noise from normal cells and from sickle cells during the delay period probably corresponds to the previously described 'flicker phenomenon', which is thought to arise from brownian motion[11–14]. Fluctuations in cell thickness from brownian motion could produce fluctuations in the measured intensity by modulating the interference between laser light reflected from the top and bottom cell surfaces. The increase in internal viscosity resulting from polymer formation would then decrease the noise by damping the thickness fluctuations.

The second type of progress curve was found for cells exhibiting apparently slow intracellular polymerization. These curves showed a sudden and pronounced decrease in the amplitude of the noise, but the change in average light scattering following the noise decrease varied enormously from cell to cell, and in repeated measurements on the same cell. We interpret these distorted kinetic progress curves as resulting from cell motion that is occurring simultaneously with polymer formation, that is, morphological 'sickling'[5–9]. For these cells the delay time was taken as the time of the decrease in noise. Visual observations showed that there was no morphological change in the cell during the delay period measured in this way.

For delay times less than 0.03 s almost all cells exhibited polymerization type progress curves, while for cells with delay times longer than about 3 s there was no increase in light scattering from polymerization following the delay period. Of the 453 cells examined, 276 (61%) exhibited solution type polymerization progress curves, 146 (32%) exhibited progress

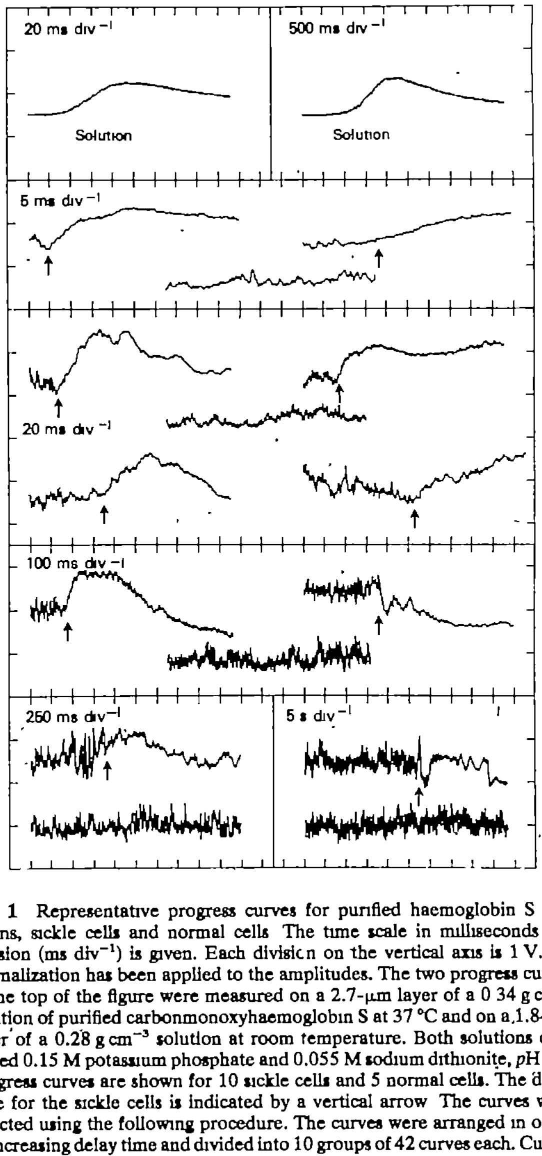

Fig. 1 Representative progress curves for purified haemoglobin S solutions, sickle cells and normal cells. The time scale in milliseconds per division (ms $\mathrm{div^{-1}}$) is given. Each division on the vertical axis is 1 V. No normalization has been applied to the amplitudes. The two progress curves at the top of the figure were measured on a 2.7-μm layer of a $0.34\ \mathrm{g\ cm^{-3}}$ solution of purified carbonmonoxyhaemoglobin S at 37 °C and on a 1.8-μm layer of a $0.28\ \mathrm{g\ cm^{-3}}$ solution at room temperature. Both solutions contained 0.15 M potassium phosphate and 0.055 M sodium dithionite, *p*H 7.1. Progress curves are shown for 10 sickle cells and 5 normal cells. The delay time for the sickle cells is indicated by a vertical arrow. The curves were selected using the following procedure. The curves were arranged in order of increasing delay time and divided into 10 groups of 42 curves each. Curves were chosen as 'representative' if they met the following criteria: (1) the delay time was within half the standard deviation from the average delay time for the group (except for the group containing cells with the longest delay times), (2) the polymerization amplitude (1.0±0.5 V) and the offset (1.8±0.9 V) were within half the standard deviation for the mean of all measurements; (3) the peak-to-peak noise before and after the delay were within 1 s.d from the mean for the group. 32 out of the 422 curves met these criteria

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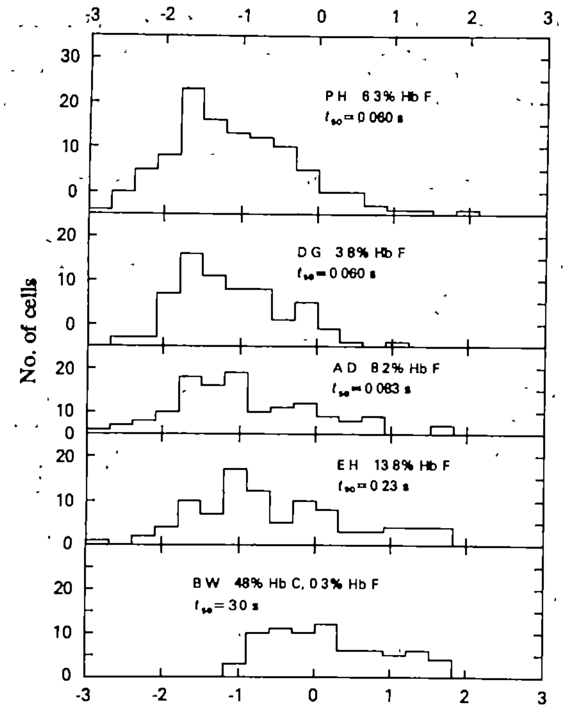

Fig. 2 Histogram of delay times on a logarithmic scale at 37 °C for red cells from four patients with homozygous SS disease and one patient with SC disease The median delay time (t_{50}) and per cent fetal haemoglobin (Hb F) measured by the alkaline denaturation method are given in the figure. The number of cells with delay times >50–100 s were 4 out of a total of 155 cells for patient P.H., 4 of 105 for D.G., 4 of 87 for A.D., 6 of 101 for E.H., and 21 of 94 for B.W. These cells were included in the calculation of the median delay time.

curves with delay times determined by the noise decrease, 13 (3%) exhibited uninterpretable progress curves and 18 (4%) exhibited progress curves similar to normal cells for 50–100 s, with no morphological evidence of intracellular polymerization at the end of this observation period. Progress curves were not recorded for more than 50–100 s, as this would have significantly slowed data collection.

An interesting difference between cells exhibiting rapid and slow polymerization was found in the reproducibility of the delay time. In a separate series of experiments progress curves were measured repeatedly on 13 cells with mean delay times ranging over 0.05 to 11 s. For the nine cells with mean delay times <3 s the percentage standard deviation from the mean was 19%, with a range of 10–36%. Measurements could be repeated an average of 23 times (range 11–30) before the morphological change produced by intracellular polymerization became irreversible, and further experiments yielded uninterpretable progress curves. For the four cells with mean delay times longer than 3 s, the measurements could be repeated on average only 11 times (range 8–14), and the delay times were much less reproducible, with an average standard deviation of 71% (range 52–99%). This irreproducibility of long delay times is also characteristic of polymerization in solution, and has been previously interpreted as arising from the stochastics of the homogeneous nucleation of single polymers in the small sample volumes (5×10^{-11} cm^3)[10].

Figure 2 gives the main results of our kinetic study, which shows the delay time histogram for the four patients with homozygous SS disease. There is an extremely wide distribution of delay times, varying from 1 ms to >100 s. This wide range of delay times was expected[1] from the known heterogeneity in intracellular haemoglobin S concentration[15] and the enormous sensitivity of the delay time of solution polymerization to initial haemoglobin S concentration (delay time is inversely proportional to the 15th to 40th power of concentration)[1,10,16]. Solution studies have shown that other variable cellular factors, such as pH[16,17] and 2,3-diphosphoglycerate levels[18,19], are relatively unimportant in determining the delay time.

To compare quantitatively the delay times observed in cells and in isolated solutions, we calculated the distribution of intracellular haemoglobin S concentrations from the delay time histogram (Fig. 3). For this calculation we used the sum of the delay time histograms for the three patients with fetal haemoglobin levels below 9% and the concentration dependence of the delay time measured on purified haemoglobin S solutions using a similar laser-photolysis light scattering technique[10]. The calculated distribution in Fig. 3 yields a mean value of 0.32 g cm^{-3} for the intracellular haemoglobin S concentration, which is in excellent agreement with the mean value of 0.30 ± 0.02 to 0.32 ± 0.02 g cm^{-3} measured by conventional methods[15,20–22]. The concentration distribution shows that the intracellular haemoglobin S concentration extends to >0.40 g cm^{-3}, in agreement with measurements on density fractionated cells[15,23] (M. R. Clark and S. B. Shohet, personal communication). The extension of the calculated distribution to very low values of haemoglobin S concentration presumably results from the cells containing a large fraction of haemoglobin

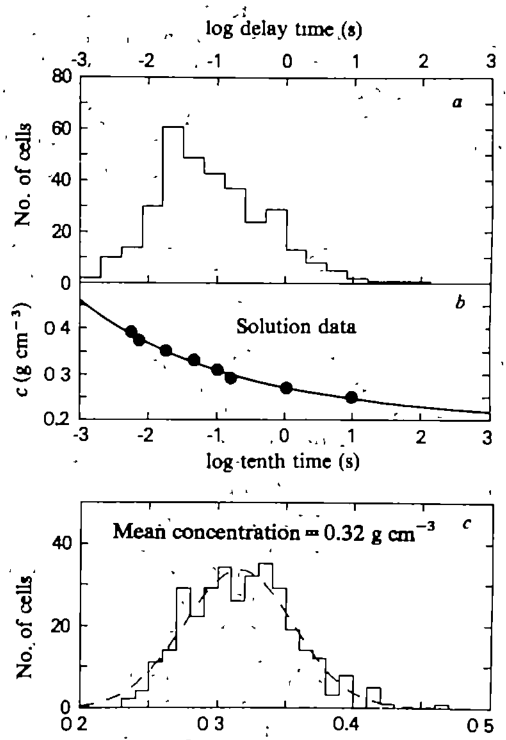

Fig. 3 Determination of the concentration distribution in sickle cells from the delay time distribution. *a*, This histogram of delay times on a logarithmic scale is the sum of the histograms from Fig. 2 for patients P.H., D.G. and A.D. The histogram from patient E.H. was not included, as the fetal haemoglobin content of 13.8% is more than 2 s.d. from the mean of 5.6 ± 3.6% found for sickle cell patients[20,21]. *b*, The concentration of purified carbonmonoxyhaemoglobin S is plotted against the logarithm of the tenth-time. The tenth-time is the time required to reach 1/10 of the polymerization amplitude, and is not significantly different from the delay time taken as the time of onset of a measurable increase in light scattering, which was the criterion used for the sickle cell progress curves. The data (filled circles) were obtained by Ferrone *et al.*[10], using the same laser-light scattering photolysis technique for purified haemoglobin S at 35 °C in 0.15 M potassium phosphate buffer, 0.055 M sodium dithionite, pH 7.1. The curve through the data is a least-squares fit of the empirical function c (g cm^{-3}) = $a\exp(-bx+dx^2)+e$, where x is the logarithm of the tenth-time in seconds, $a=0.104$, $b=0.287$, $d=0.021$ and $e=0.167$). In carrying out the fit the data points were weighted according to the reciprocal of their standard errors. *c*, The histogram of intracellular haemoglobin S concentrations was obtained by calculating the concentration corresponding to each delay time with the empirical function used to draw the curve in *b*. The data are reasonably well represented by a gaussian function (dashed curve), $P=a\exp(-(b-c)/d)^2$, where c is the intracellular haemoglobin S concentration in g cm^{-3}, $a=33.5$, $b=0.316$, $d=0.057$. The detailed shape of the concentration distribution is least certain above 0.40 g cm^{-3}, where there are no solution data.

F[24], which is known to increase the delay time markedly[3,25]. The high fetal haemoglobin content in the cells from patient E.H., moreover, is most probably responsible for the shift of the delay time distribution (Fig. 2) to longer times.

The close correspondence of the calculated and measured concentration distributions, the similarity in shape of the cell and solution polymerization progress curves, and the irreproducibility of the long delay times in cells together argue strongly that polymerization of haemoglobin S inside cells proceeds by the same nucleation and growth mechanism as in purified solutions. The results of a previous kinetic study, which showed that red cell membrane components have no significant effect on the delay time of purified solutions[26], considerably strengthen this conclusion.

Delay time measurements on cells should provide a sensitive method for assaying potential therapeutic agents. To evaluate the results, however, it will first be necessary to establish criteria for effectiveness by measuring delay time distributions for sickle cell syndromes of varying clinical severity. As an example, we have measured the distribution of delay times from a patient with sickle cell haemoglobin C (SC) disease (Fig. 2). The median delay time (t_{50}) is 3 s, much longer than the t_{50} of 0.06–0.23 s for the SS patients (Fig. 2). The delay time distribution found for SC cells is consistent with the clinical finding that SC disease is intermediate in severity between SS disease and the essentially benign sickle cell trait[20], in which the delay times are much longer[7,27].

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Protein dynamics and NMR relaxation: comparison of simulations with experiment

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Comparisons between molecular dynamics simulations of proteins and experiment have been based on temperature factors[1–5] derived from X-ray spectra and on the stability of hydrogen bonds[6]. Here we present a novel method of testing molecular dynamics simulations against nuclear magnetic resonance (NMR) relaxation measurements, based on the recently developed model-free approach[7] to the interpretation of NMR data. As NMR relaxation in proteins is determined by dynamics on the picosecond–nanosecond time scale, a comparison of NMR experiments and molecular dynamics simulations that last for less than ~100 ps is more meaningful than previous ones[3–6] involving much longer time scales. We make contact between a molecular dynamics simulation[8] and a ^{13}C-NMR relaxation study[9] of pancreatic trypsin inhibitor (PTI) by comparing generalized order parameters (which are measures of the extent of angular motion of the bonds) extracted from the relaxation data[9] with those calculated[10] from a 96-ps trajectory[8]. We show that the theoretical order parameters indicate less motion than their experimental counterparts. The relative flexibility of the residues studied, however, is reasonably well described by the simulation.

At presently available spectrometer frequencies, ^{13}C-NMR relaxation of protonated carbons is determined by the reorientation of the ^{13}C—H bond vectors relative to the external magnetic field. For a protein in solution both the motion of the macromolecule as a whole and internal motions in a macromolecule-fixed frame contribute to the relaxation. It has been shown recently[7] that the unique information on fast internal motions contained in NMR relaxation experiments can be specified by two model-independent quantities: an effective correlation time, τ_e, and a generalized order parameter, $\mathcal{S}$. These quantities can be extracted from relaxation data using a simple fitting procedure[7]. τ_e is proportional to the area of the time correlation function for internal motions; the time correlation function can have components which decay with correlation times faster or slower than τ_e. For ^{13}C-NMR of protonated carbons, $\mathcal{S}$ is a measure of the angular restriction of the motion of a ^{13}C—H vector and is defined as

$$\mathcal{S}^2 = \frac{4\pi}{5} \sum_{m=-2}^{2} |\langle Y_{2m}(\Omega)\rangle|^2 \tag{1}$$

where Y_{2m} are spherical harmonics, Ω specifies the orientation of a ^{13}C—H bond in a macromolecule-fixed frame and the angular brackets denote an average over all possible orientations accessible on a time scale shorter than the correlation time for the overall reorientation of the macromolecule (~4 ns for PTI). It follows from equation (1) that $0 \leq \mathcal{S}^2 \leq 1$ and that $\mathcal{S} = 0$ when the internal motion is isotropic, while $\mathcal{S} = 1$ when the motion is completely restricted.

Values of $\mathcal{S}$ and τ_e of ^{13}C—H bonds for 12 methyl groups have been extracted[7] from the relaxation data of Richarz *et al.*[9]. The τ_e values which contain contributions due to motions of and about the symmetry axis of the methyl groups, were in the range 19–70 ps.

As in the molecular dynamics simulation of Karplus and McCammon[8] the hydrogens are incorporated into the heavy atoms to which they are bound, it is impossible to calculate $\mathcal{S}$ and τ_e for methyl groups directly from their trajectory. However, a comparison with experimental order parameters becomes possible if it is assumed that the motion of the methyl symmetry axis (for example, the C$^\alpha$—C$^\beta$ bond in alanine) and the motion of the protons about this axis are uncoupled. In this

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[*] Deceased 19 June 1982

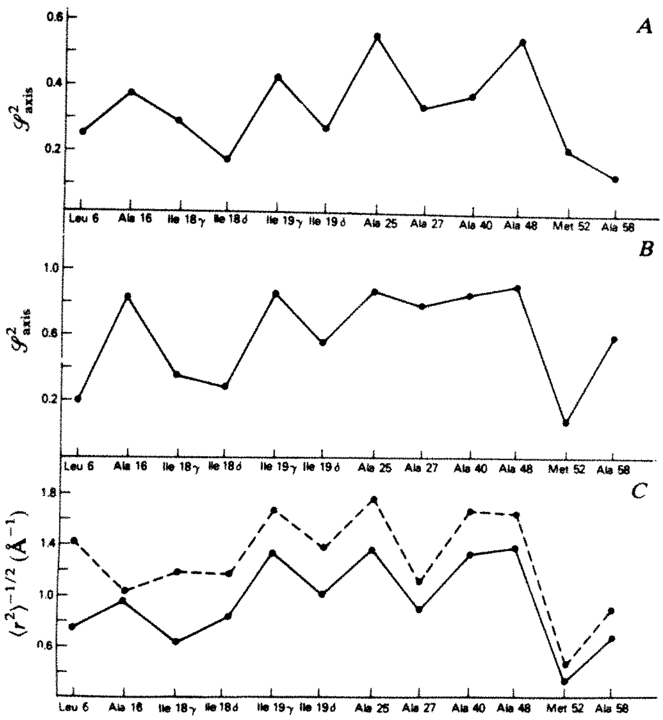

Fig. 1 Comparison of experimental order parameters (*A*) with those calculated from a molecular dynamics simulation (*B*). *C* shows the reciprocals of the theoretical r.m.s. positional fluctuations for the methyl carbons (solid line) and the adjacent heavy atoms (broken line). The experimental order parameters for the δ-carbons of Ile 18 and Ile 19 are based on our assignment of the resonances (see text).

case, independent of the nature of the motion of the symmetry axis, one has[7]

$$\mathcal{S}^2 = \mathcal{S}^2_{\text{axis}}\left(\tfrac{1}{2}(3\cos^2\beta - 1)\right)^2 \qquad (2)$$

where β is the angle between the ^{13}C– H bond and the symmetry axis and $\mathcal{S}_{\text{axis}}$ is the generalized order parameter of the bond joining a methyl carbon and its nearest-neighbour heavy atom. $\mathcal{S}^2_{\text{axis}}$ can be calculated from a trajectory by replacing the average in equation (1) by a time average.

Figure 1*A* shows values of $\mathcal{S}^2_{\text{axis}}$ obtained from the values of $\mathcal{S}^2$ extracted[7] from the experimental relaxation data[9] using equation (2) with the value of β (69.8°) determined from a neutron diffraction study[11] of alanine. The experimental relaxation parameters for Ala 16 and Ala 40 are average values because these resonances were not resolved (we used the same order parameters for these two residues). The δ-carbon resonances of Ile 18 and Ile 19 could be identified (resonances *a* and *b*) but were not assigned[9]. In constructing Fig. 1*A* we assigned resonance *a* to the Ile 18 δ-carbon so that the relative ordering of $\mathcal{S}^2_{\text{axis}}$ of the δ-carbons of Ile 18 and Ile 19 agrees with the simulation (Fig. 1*B*). Figure 1*B* shows the values of $\mathcal{S}^2_{\text{axis}}$ calculated from a 96-ps trajectory of PTI[8]. With the exceptions of Met 52 and Leu 6, the theoretical order parameters are larger than the experimental ones, indicating that there is usually less motional averaging in the 96-ps simulation than detected in the experiment. Such behaviour will occur when the length of the trajectory is too short for a bond to assume all accessible orientations. If there are activation barriers between various conformations, the time required for a single computer simulation to develop an equilibrium distribution of bond orientations will probably be longer than 100 ps (that is, the trajectory can be trapped in a local minimum). The very small order parameter (0.069) for the S—C$^\epsilon$ bond of Met 52 calculated from the trajectory seems to be due to the neglect of solvent effects in the simulation. Recently, it has been shown[5] that the inclusion of solvent leads to a dramatic reduction of the average of r.m.s. displacements of Met 52. Based on the experimental order parameters, we conclude that the motion of the C$^\alpha$—C$^\beta$ bond of Ala 58 is the least restricted of all the residues studied. According to the theoretical order parameters, however, Ala 58 is the most mobile only among the alanines. It is possible that the experimental order parameter of Ala 58 is inaccurate as it is based on only two measured relaxation parameters whereas the other order parameters, with the exception of those of Ala 16 and 40, are based on three. Although differences do exist, it can be seen from Fig. 1*A*, *B* that the relative flexibility of the residues is well described in the simulation. For example, the relative mobilities of all the alanines ($58 > 40 \simeq 27 \simeq 16 > 25 \simeq 48$) is predicted correctly.

Using the model-free approach, an order parameter of 0.87 was extracted from the relaxation data for the α-carbon envelope of PTI. This order parameter, determined by the reorientation of the C$^\alpha$—H vectors, is a measure of the average local flexibility of the backbone. Since to a first approximation the C$^\alpha$—C$^\beta$ bonds are expected to move like the C$^\alpha$—H vectors, we evaluated the order parameters of the C$^\alpha$—C$^\beta$ axis for all protonated α-carbons in PTI. The average was 0.84, very close to the experimental value. Note, however, that the experiment indicates that the α-carbons of alanines move more than the average of all the α-carbons (that is, $\bar{\mathcal{S}}^2_{\text{axis}} = 0.39$ which is less than 0.87) whereas the molecular dynamics predicts that the motion of the alanines is close to the average (that is, $\bar{\mathcal{S}}^2_{\text{axis}} = 0.81$ as opposed to 0.84).

Finally, it is of interest to compare the pattern of the values of the order parameter to the trends of isotropic r.m.s. displacements from the average atomic positions for a series of residues. As $\mathcal{S}^2_{\text{axis}}$ depends on the concerted motions of two nuclei, in Fig. 1*C* we show the reciprocals of the r.m.s. displacements of both the methyl carbons and their nearest neighbours calculated from the 96-ps trajectory. We have plotted the reciprocals to facilitate a visual comparison with the order parameters. In general, there need not be a simple connection between the ordering of $\mathcal{S}^2$ and the r.m.s. fluctuations as these quantities reflect motional flexibility in different ways. For example, although the calculated order parameters of Ala 16 and 40 are almost the same, the r.m.s. fluctuations of Ala 16 are greater than those of Ala 40. Nevertheless, by comparing Fig. 1*B* and *C* it can be seen that there exist many similarities between the pictures of the relative mobility of residues in a protein derived from order parameters and those derived from r.m.s. fluctuations (for example, Ile 18 is more mobile than Ile 19, and Ala 27 is more mobile than Ala 25 by both criteria).

We have described a novel means of testing molecular dynamics simulations where properties related to motions about individual bonds are compared with their experimental counterparts. To our knowledge, this is the first time that the results of a simulation have been related to observable properties at this level of detail. As more experimental data become available on a variety of protein systems, our approach should prove useful in assessing the range of validity of molecular dynamics simulations.

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MATTERS ARISING

Simulating silicate structures and structural chemistry of pyroxenoids

CATLOW *ET AL.*[1] have recently described a way of calculating lattice energies of different silicate structures in an attempt to predict which single-chain silicate structure would be the most stable for a particular metal cation or for a particular pair of cations. The stabilization energy (the difference of lattice energies calculated) of one structure with respect to another is often a very small fraction (~0.1%) of the total lattice energy (calculated per Si atom) and therefore a considerable degree of confidence in the values calculated is required.

The essentially ionic model presented has an empirical correction for 'short-range' interaction—presumably for orbital overlap or covalency effects. This correction does not appear to take account of the fact that in some cation environments not all oxygen ligands are equivalent; some oxygens are so-called bridging oxygens between silica tetrahedra and some are directly bonded to one Si atom only. Whether the neglect of this variation in short-range interaction is significant enough to cause appreciable error is not clear.

Nevertheless, there are many further tests Catlow *et al.* could make to clarify the quantitative validity of their model. For example, it is misleading to conclude that the most energetically favoured pyroxene or pyroxenoid structure for Mg^{2+} cations is the diopside structure when it is known that a pure Mg pyroxene has an ortho- or clino-enstatite structure and not a diopside structure. A lattice energy calculation for Mg^{2+} in an ortho- or clino-enstatite structure should therefore show that the latter structures are more stable than the diopside one. (The same argument applies to Fe^{2+}.)

However, the most intriguing point is whether such lattice energy calculations are refined enough to predict cation ordering correctly in such silicates. There is now enough experimental evidence of cation ordering (or the lack of it) in olivines and pyroxenes to provide a testing ground for such calculations. Traditionally explanations of cation ordering have been on the basis of ionic size or, in the case of transition metals, in terms of crystal field stabilization effects. Such explanations are sometimes the result of hindsight and are often less than convincing; sometimes they fail altogether. Clearly therefore it would be very useful to be able to carry out an energy calculation from first principles. Such calculations ought then to be able to predict for example that Fe^{2+} is not ordered in the olivine structure but that Mn^{2+} has a strong preference for the M_2 site—putting Mn^{2+} in the M_2 site rather than the M_1 site should lead to greater stability whereas no appreciable stabilization should occur for Fe^{2+}.

Catlow *et al.* show that the effect of having mixed cations often has a stabilization effect on a particular structure. However, the maximum stabilization will, of course, only be achieved if the correct distribution of cations among the possible sites is chosen. Calculations with different cation distributions should therefore predict the correct one.

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1. Catlow, C R. A., Thomas, J M., Parker, S C. & Jefferson, D A. *Nature* **295**, 658–662 (1982)

CATLOW AND PARKER REPLY—The comments of Walker on our simulation study of pyroxenoids raise two issues. First, what is the 'accuracy' of the calculated lattice energies; second, what further tests of our method are possible. Regarding the question of accuracy we stress that the methods used by our calculations are exact; there are no errors (above the level of machine accuracy) attributable to our lattice summation techniques. Any 'inaccuracy' arises from inadequacies in our interatomic potentials. Here we note that our potentials perform well in predicting structures of the type of mineral discussed in our article, which supports their reliability in a study of structure discrimination. Moreover, we note that the differences between the energies of different structures are appreciable (that is ~1 eV). The fact that these are a small proportion of the total energy does not mean that they are insignificant; indeed the total energy contains large terms which are relatively insensitive to the structure. The position here is analogous to that in quantum mechanical studies of the energies of small molecules, where binding energies, although a small proportion of the total energy may, nevertheless, be reliably calculated. The point raised by Walker concerning the possibility of different potential parameters for bridging and non-bridging oxygen atoms is valid. However, the success of our calculations in accurately predicting structures suggests that this is not a major effect, although refinements of our potentials should clearly take this effect into account.

As further tests of our calculations Walker suggests first that we examine the enstatite structure of $MgSiO_3$ which he correctly remarks is the most stable structure of this phase. Such calculations are indeed in progress; and our results find that the enstatite structure is more stable than the diopside structure for $MgSiO_3$ and $FeSiO_3$; further details of these calculations will be reported shortly. Walker also suggests that we use our techniques to tackle problems relating to cation ordering. It was indeed with the longer-term view of investigating such problems, that we originally developed our simulation methods. The success of our work encourages the extension to the problems discussed by Walker, although we emphasize that the computational demands of such work are considerable.

Finally, we should point out that the calculations we reported had the main aim of investigating whether energy minimization techniques could usefully be applied to silicate structural chemistry. We believe that our work established the viability of the technique in this field. Subsequent studies will aim at refining our potential models and hence the accuracy of our predictions.

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Chemi-ionization in acetylene flames

EVIDENCE has been presented by Hayhurst and Jones[1] in favour of the preponderance of the reaction

$$CH^* + C_2H_2 \rightarrow C_3H_3^+ + e^-$$

over the reaction

$$CH + O \rightarrow CHO^+ + e^-$$

as the primary ionization process in rich acetylene/oxygen flames. This evidence is: (1) low relative concentration of CHO^+, particularly in the reaction zone; (2) double-peaked profile of $C_3H_3^+$ in rich C_2H_2/O_2 flames; (3) double-peaked profile of total ion current in rich C_2H_2/O_2 flames.

All these features may, however, be explained by a mechanism based on CHO^+ alone. It has been realized since the earliest work on mass spectroscopy of flame ions[2] that rapid proton transfer reactions will deplete CHO^+ in flames so that its concentration relative to prominent ions such as H_3O^+ (from protonation of water) will be low. Computer simulations of the kinetics of a simple proton transfer scheme have shown that CHO^+, although the primary ion, will be removed by reaction with H_2O in 10^{-6}–10^{-5} s (ref. 3). The double-peaked feature in the profile of $C_3H_3^+$ is also produced by computer simulation of the scheme:

$$CHO^+ + H_2O = H_3O^+ + CO \qquad (1)$$
$$k_f = 2.5 \times 10^{-9} \quad cm^3\,s^{-1}$$
$$k_r = 5.6 \times 10^{-13} \quad cm^3\,s^{-1}$$

$$CHO^+ + C_3H_2 = C_3H_3^+ + CO \qquad (2)$$
$$k_f = 10^{-9} \quad cm^3\,s^{-1}$$
$$k_r = 1.8 \times 10^{-18} \quad cm^3\,s^{-1}$$

$$H_3O^+ + C_3H_2 = C_3H_3^+ + H_2O \qquad (3)$$
$$k_f = 10^{-9} \quad cm^3\,s^{-1}$$
$$k_r = 4 \times 10^{-15} \quad cm^3\,s^{-1}$$

in which the concentration of C_3H_2, of the order of 10^{-6} mol fraction, is made to follow a double-peaked profile. Reaction (2) was first suggested by Hayhurst and Kittleson[4]. C_3H_2 is an entity about which little is known beyond theoretical calculations of heat of formation, which indicate that it may have a high proton affinity[5,6]. If it occurs in the reaction zones of acetylene flames in association with CH, it may well also recur in the downstream feather described by Hayhurst and Jones, in association with CH and polyacetylene molecules. Furthermore, there are several ion/molecule reactions involving acetylene and other hydrocarbons which produce $C_3H_3^+$ (ref. 7).

Finally, the double-peaked profile for the total ion current could be due to a fresh downstream production of CHO^+ (from CH generated in the 'feather') which would then rapidly generate $C_3H_3^+$ by the mechanism described above.

These points demonstrate the difficulty of finding proof of one mechanism of flame ionization as opposed to another, and the need fully to recognize alternatives in the interpretation of experimental results.

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HAYHURST AND JONES REPLY—We accept "the need fully to recognize alternatives in the interpretation of experimental results" and in this spirit suggested[1] that

$$CH^* + C_2H_2 \rightarrow C_3H_3^+ + e^- \qquad (2)$$

produces ions in addition to the well-established process:

$$CH + O \rightarrow CHO^+ + e^- \qquad (1)$$

in very fuel-rich oxy-acetylene flames. Our proposal is based primarily on one piece of evidence, that the profile of total positive ion concentration along such a flame's axis displays two maxima. This indicates that there are two spatially separated regions of ion production. McAllister's explanation of this observation is based on both C_3H_2 and ground state CH having double-peaked profiles. The available evidence[2,3] indicates that the concentrations of C_xH_y intermediates in these flames show only one maximum. In any event, McAllister's scheme involving C_3H_2 is not the only route[4,5] from CHO^+ to $C_3H_3^+$ and does not address itself to the generation of ions from neutrals.

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature.* The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 500 words and the briefest of replies, to the effect that a point is taken, should be considered.

Little quantitative information is available on neutral species in these fuel-rich flames. However, it is known[6] that the concentration of oxygen atoms is low in the reaction zone and rises to a maximum just before the end of the feather, that is, close to the second maximum in the rate of production of ions, which we attribute[1] to reaction (1). It is likely[3] that ground state CH displays one maximum in its concentration somewhere in the feather, but its exact location is not known. Electronically excited CH is possibly unusual[6] in displaying two concentration maxima—one where the rate of ion production first maximizes and another farther downstream in the feather. Such complications no doubt derive from CH* being produced[3] by $C_2 + OH \rightarrow CH^* + CO$. The concentration of C_2H_2 declines[6] steadily through the feather. It is thus clear that the product $[CH^*][C_2H_2]$ has a maximum in the reaction zone, whereas [CH] [O] has one farther downstream. This is in line with our suggestion[1] that the first ions are produced by reaction (2), but subsequent ones are generated by reaction (1).

The area of any disagreement thus centres on the process responsible for the original ions in the reaction zone of one of these fuel-rich flames of C_2H_2. Notably, only one oxygenated ion ($C_2H_3O^+$, probably protonated ketene) is detected in the reaction zone. The main positive ion is $C_3H_3^+$ and in fact all the other ions have the formula $C_xH_y^+$. The apparent absence of oxygenated ions, such as H_3O^+ and CHO^+, suggests that the reactions proceeding in the reaction zone and early part of the feather are largely the pyrolysis of hydrocarbon molecules and radicals, rather than their oxidation, which occurs further downstream[7]. This agrees with the observation[6] that oxygen atoms have low concentrations in the reaction zone.

The ratio of the rates of reactions (1) and (2) is $k_1[CH]\,[O]/k_2[CH^*]\,[C_2H_2]$. The value of k_1/k_2 can be estimated to be around unity. [CH*] is much higher than expected on thermodynamic grounds[3] and can be expected to have a mol fraction in these reaction zones of $\sim 10^{-8}$. Mol fractions of 0.1 and 10^{-4} can be estimated for C_2H_2 and CH, respectively. This means that for reaction (1) to dominate reaction (2) in the first production of ions, oxygen atoms must have a mol fraction exceeding 10^{-5}, which is most unlikely[8]. Alternatively, the initial rate of production of ions is measured from our[1] Fig. 1 to be around 3×10^{14} ions $ml^{-1}\,s^{-1}$. If only reaction (2) operates, then the above [CH*] and $[C_2H_2]$ indicate $k_2 \sim 3 \times 10^{-13}$ ml per ion per s, which is entirely reasonable. Note that k_1 has been measured[9] to be 2.8×10^{-13} ml per ion per s at the similar temperature of 2,200 K.

Of course, the above discussion in favour of reaction (2) does not prove that it operates. It does indicate, however, that reaction (2) must be taken as a serious possibility in fuel-rich acetylene flames, where conditions are most favourable for it.

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ANNOUNCEMENTS

Appointments

His Royal Highness The Prince of Wales has been elected to Honorary Fellowship of The Institution of Electrical Engineers.

Two appointments have been made at Queen's University, Belfast, **Dr John Blakeman** has been elected to the Chair of Mycology and Plant Pathology, and **Mr Anthony Valentine** has been appointed to the Chair of Paediatric and Preventive Dentistry.

Dr Bruce A. Reitz, a Stanford University National Science Foundation will take up the position of Chancellor of the College Park campus of the University of Maryland, USA.

Dr Bruce A. Reitz, a Standord University heart surgeon, will become professor and director of the Division of Cardiac Surgery at The Johns Hopkins School of Medicine, Baltimore, USA and cardiac surgeon in charge at The Johns Hopkins Hospital.

Douglas A. Fenderson, director of continuing medical education at the University of Minnesota, has been appointed director of the National Institute for Handicapped Research, USA.

Action on Smoking and Health (ASH) announced that **Professor Peter Sleight,** Field Marshal Alexander Professor of Cardiovascular Medicine at Oxford University, has been appointed its new Chairman.

Dr P W Allen, Assistant to the Director, Malaysian Rubber Producers' Research Association, has been appointed Secretary of the International Rubber Research and Development Board UK.

Dr W. Rizk has been appointed Chairman of the Board of The British Standards Institutions (BSI). He succeeds Mr Paul Fletcher who has retired after completing a three year period of office.

Professor Yadin Dudai has been named first incumbent of the Michael and Sara Sela chair in Neurobiology, recently established at the Weizmann Institute.

Dr Robert Lickley has been elected into Honorary Fellowship of the Institution of Mechanical Engineers.

Maximilian Buja, professor of pathology at The University of Texas Health Science Center at Dallas USA was named the first A.J. Gill Professor of Pathology at the health science center.

The Open University has appointed **Roger Mills** as director of its London regional office.

Awards

The Serono Prize in Steroid Endocrinology for 1982 was presented to **Mr Aristidis Slavakis**, a Greek student, on the MSc Course in Steroid Endocrinology at Leeds University, UK.

The British Society of Rheology has made its Annual Award for 1982 to **Professor F.M. Leslie** of the University of Strathclyde in recognition of his contributions towards an understanding of the rheology of liquid crystals.

Dr Karl Frank Austen, of Harvard Medical School, USA, and **Dr Bengt Ingemar Samuelsson** of the Karolinska Institute in Stockholm, Sweden have been selected to receive the 1982 Waterford Biomedical Science Award for their work in allergy and inflammation.

The international award in the BP Energy Research Prize competition has been awarded to **Dr R.P. Howson,** department of physics, Loughborough University of Technology, for his project on selective optical coatings on plastic sheets.

Mr David Attenborough has been awarded the 1981 Kalinga Prize for the Popularization of Science jointly with **Mr D. Flanagan,** editor of *Scientific American.* The award was established by UNESCO in 1951 by means of a grant from the Kalinga Foundation Trust.

Professor H.H. Hopkins, Professor of Applied Optics in the University of Reading, has been awarded the 1982 Gold Medal of the International Society for Optical Engineering for his contributions spanning a broad range of theoretical and applied optical disciplines.

Engelhard Corporation of Iselin, New Jersey USA has been presented with the Council of Environmental Quality's first 'Award of the Decade' for environmental innovation and achievement by American industry. Engelhard was also cited by the United Nations for its contributions to environmental protection.

The Science and Engineering Research Council (SERC) has awarded a special grant of £350,700 for the study of transport and kinetic processes in fixed catalytic reactors. This work, to be undertaken by **Prof. C. McGreavy** (University of Leeds) and **Prof. W.J. Thomas** (University of Bath), is expected to lead to improvements in the design of catalytic reactors.

Whatman Reeve Angel Plc, of Maidstone, Kent, UK., who produce Laboratory Filter and chromatographic Media, are the winners of the 1982 Sedgwick Safety Award for the South East Region.

The Paul-Martini-Prize, founded in order to promote the development of scientific methods for evaluation in the clinical-pharmacological and therapeutical field, was shared 1982 by three German Scientists — **Prof. Dr Gustav Georg Belz** of the Institut for Kardiovaskuläre Therapie in Weisbaden, **Dr Wittich Doering** of the II. Medizinische Klinik des Krankenhauses München-SChwabing and **Dr Jürgen Eberhard Scherberich,** Zentrum der Inneren Medizin at the Klinikum der Johnann Wolfgang-Goethe-Universität, Frankfurt.

The Wellcome Trust has announced the 10 new awards of Wellcome Trust Senior Lecturerships; **Dr Jenefer M. Blackwell,** Ross Institute of Tropical Hygiene, London School of Hygiene & Tropical Medicine; **Dr S.G. Cull-Candy,** Department of Pharmacology, University College London; **Dr P.M. Dean,** Department of Pharmacology, University of Cambridge; **Dr Jean Golding,** Department of Child Health, University of Bristol; **Dr D.J. Harvey,** Department of Pharmacology, University of Oxford; **Dr R.F. Hess, The Physiological Laboratory, University of Cambridge; Dr Hazel C. Jones,** Department of Zoology, University of Hull; **Dr M.C. Sheppard,** Department of Medicine, University of Birmingham; **Dr D.G. Thompson,** Medical Unit, The London Hospital Medical College; **Dr H.V. Wheal,** Department of Neurophysiology, University of Southampton.

The prizewinners of the Feldberg Foundation awards for 1982 are **Professor D. Oesterhelt,** of the Max Planck Institute for Biochemistry, Munich Germany and **Dr G. Radda** of the Department of Biochemistry, University of Oxford, UK. The Feldberg Foundation for German-English Scientific Exchange aims to promote Anglo-German friendship in the medical and biological sciences.

John H. Warden of the Water Research Centre, Stevenage, UK has been awarded the major share of this year's $10,000 Chemviron Award for environmental improvement. Other winners are two professors from the University of Hanover in Germany, **Dr H. Ruffer and Mr T. Slokman.**

Pfizer Central Research have announced that they will make up to six new awards annually of £1,500 each to support young scientists who have carried out meritorious research at British Universities. The work should have potential application in the search for new human or animal health drugs. Two awards will be made available for research in Organic Chemistry, two in Biology, one in Pharmaceutical Sciences and one in Veterinary Research. Further details from: Miss M. Kitney, Pfizer Ltd., Sandwich, Kent. CT13 9NJ, UK.

Entries are invited for a competition to design and construct a voice-controlled robot. The British Computer Society is running a Voice-Controlled Robot Competition is aimed at technicians following TEC HC/HD courses in colleges and polytechnics. The competition is being organized in two stages. Stage one involves production of plans by the end of December 1982. Six designs will then be selected for construction in the second stage, which will conclude in July 1983.

BP PLC, the largest British oil company, has provided the Royal Society, UK with the sum of £30,000 for expenditure over the next twelve months on awards to enable Chinese and British scientists to work side by side, either in joint research projects, or for visits in either direction associated with scientific discussions or seminars, contributing to Sino-British scientific interchange.

Applications are invited for the Rolex Awards for Enterprise 1984. Projects must display a true spirit of enterprise, and fall into the categories of Applied Sciences and Invention, Exploration and Discovery or The Environment. There are five prizes, each of 50,000 Swiss Francs and a gold Rolex chronometer. Application forms from: The Secretariat, The Rolex Awards for Enterprise, PO Box 178, 1211 Geneva 26, Switzerland, before 31 March 1983.

Meetings

3-24 January, **Training Course on Molecular Aspects of Developmental Biology,** Puerto Rico (B.C. Candelas, Dept of Biology, University of Puerto Rico, Rio Piedras, Puerto Rico 00931, USA).

17-20 January, **The Biology and Biochemistry of Cellular Interactions,** Siena (Dr C. Ceccarini, Centro Ricerche, ISVT Sclavo, Via Fiorentina 1, 53100 Siena, Italy).

17-21 January, **Advances in Gene Technology: Molecular Genetics of Plants and Animals,** Miami (Miami Winter Symposium, PMBA Conference, PO Box 016129, Miami, Florida 33101 USA).

20 January, **Institution of Mining and Metallurgy,** London (The Institution of Mining and Metallurgy, 44 Portland Place, London W1N 4BR, UK).

28 January, **The Challenger Society,** London (The Challenger Society, Institute of Oceanographic Sciences, Wormley, Godalming, Surrey. GU8 5UB, UK).

22-24 November. The, meeting on **Cytopathology of Parasitic Disease** is to be held in Caracas, not London as stated in 7 October issue, (The Ciba Foundation, 41 Portland Place, London W1, UK.)

14-18 February, **Safety Training Programme,** Loughborough (The Centre for Extension Studies (KG), Loughborough University, Loughborough, Leics LE11 3TU, UK).

18-23 February, **Chemtech and ORT '83 Exhibition and Conference,** India (Chemtech Secretariat, 210, Dr D.N.Road, Bombay 400 001, India).

22-23 February, **Conference on Cleaner Technologies,** London (Miss Lesley Claff, Oyez Scientific and Technical Services Ltd., Bath House (3rd Floor), 56 Holborn Viaduct, London EC1A 2EX, UK).

21-22 March, **Actions and Interactions of GABA and Benzodiazepines,** London (Mrs Joan Kruger, Department of Pharmacology, University Collge, Gower Street, London WC1E 6BT, UK).

26-30 March, **Eighteenth International Symposium on Computer Applications in the Mineral Industries,** London (The Conference Office, Institution of Mining and Metallurgy, 44 Portland Place, London W1N 4BR, UK).

27-30 March, **First European Symposium on Radiopharmacy and Radiopharmaceuticals,** Elanore, Denmark (Isotope Pharmacy, Frederikssundsvej 378. DK 2700 Copenhagen, Bronsho, Denmark.

28-31 March, **Low Energy Ion Beams,** Loughborough (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

30-31 March, **Universities Federation Animal Welfare/Laboratory Animal Science Association Joint Symposium,** London (Dr H.B. Waynforth, The Animal Unit, Charing Cross Hospital Medical School, St Dunstan's Road, London W6 8RP, UK).

10-17 April, **Environment Impact Assessment Symposium,** Crete (PADC Environmental Impact Assessment and Planning Unit, Department of Geography, University of Aberdeen, Aberdeen, Scotland, UK).

11-13 April, **Annual Chemical Congress,** Lancaster (Dr J.F. Gibson, The Royal Society of Chemistry, Burlington House, London W1V 0BN, UK).

11-15 April, **Symposium on European Rocket and Balloon Programmes and related research,** Interlaken (The Secretariat for the Programme Advisory Committee on the ESRANGE Special Project, European Space Agency, D/OPS-HQ, 8/10 rue Mario Nikis, 75738 Paris Cedex 15, France).

12-15 April, **Conference on Stabilisation and Disinfection of Sewage Sludge,** Manchester. (Mr E. Porter, Water Research Centre, Communications Group, Medmenham Laboratory, PO Box 16, Henley Road, Marlow, Bucks SL7 2HD, UK).

12-15 April, **Electrostatics Conference,** Oxford (The Static Electrification Group, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

13-15 April, **Static Electrification,** Oxford (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 1QX, UK).

14-15 April, **British Institute of Radiology's Annual Congress and Scientific Exhibition,** London (British Institute of Radiology, 36 Portland Place, London W1N 3DG, UK).

13-15 April, **International Colloquium on Diatomic Molecules,** Oxford (W.G. Richards, Physical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ, UK).

17-19 April, **Symposium on Aquatic Toxicology,** Milwaukee (M.B. Cooper, 1916 Race Street, Philadelphia, Pennsylvania 19103, USA).

18-19 April, **Symposium on the Determination of Radionuclides in Environmental and Biological Materials,** Teddington (Mr B.S. Marshall, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London SE1 9NQ, UK).

18-20 April, **Intramolecular Kinetics,** Warwick (Prof. J.P. Simons, Department of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD, UK).

18-22 April, **6th Tandem Conference,** Chester (Mrs S.A. Lowndes, Science and Engineering Research Council, Daresbury Laboratory, Warrington, Cheshire WA4 4AD, UK).

19-21 April, **International Workshop for Atmospheric Spectroscopy,** Oxfordshire (Dr R.W. Saunders (IWAS), Rutherford Appleton Laboratory, Chilton, Didcot, Oxon. OX11 0QX, UK).

19-21 April, **Physical Modelling of Multi-Phase Flow,** Coventry (The Organising Secretary, Physical Modelling of Multiphase Flow, BHRA Fluid Engineering, Cranfield, Bedfore MK43 0AJ, UK).

19-23 April, **Energy '83,** Hamburg (Hamburg Messe und Congress GmbH, Jungiusstrasse 13 Messenhaus, Postfach 3023 60, D-2000 Hamburg 36, Germany).

26-28 April, **Biologic Diagnosis and Treatment of Malignacies,** Geneva (Dr. W. Hennessen, Scientific Secretary of IABS, Willadingweg 37, CH-3006 Ben, Switzerland).

23-28 April, **The Science of Biological Specimen Preparation for Microscopy and Microanalysis,** Traverse City (Om Johari, Director, SEM Meetings, PO Box 66507, AMF O'Hare, IL 60666, USA).

24-30 April, **Euchem Conference,** Lucerene (Professor J.E. Baldwin, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, UK).

25 April-1 July, **Understanding Climte Course,** Norwich (Dr T.M.L. Wigley, Climatic Research Unit, University of East Anglia, Norwich, NR4 7TJ, UK).

27-30 April, **3rd Sitges Conference on Protein Nucleic Acid Interactions,** Sitges (Prof. J.A. Subirana, Escuela T.S. Ingenieros Industriales, Diagonal, 647, Barcelona (28), Spain).

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What Mr Andropov should do for science

Mr Leonid Brezhnev's death, and Mr Yuri Andropov's succession as the fifth holder of his party post since the Revolution, is also an opportunity for reforming the framework of Soviet science.

For the next few days, Mr Yuri Andropov will have a great deal on his mind. His first need, as one recently emerged from the grey uniformity of the Central Committee (Politburo), is to let the world know that he is flesh and blood. Last Monday's funeral oration was a start. Before turning to matters of substance, Mr Andropov will probably also follow other newly appointed national leaders in replacing the holders of posts with which he must work closely by people who are congenial to him. This will for some time be the chief source of interest to those who make a profession of watching the Soviet Union closely. Meanwhile as Andropov must know well enough, the bureaucracy will look after the day to day business for him. Yet eventually his attention must turn to the issues that occupied his predecessor — East-West relations (poor, even with the lifting of the US sanctions against West European allies for alleged transgressions on the Siberian pipeline), the state of the Soviet economy (parlous) and the state of security and the armed services — and the cost thereof. Will Andropov have the wit to escape this arid agenda to tackle the question none of his predecessors since Lenin (Khrushchev may have been a wayward exception) has had time for — the condition of Soviet science? The beneficiaries of such a departure from immediately past precedent would include not merely Andropov himself, his government, the Soviet scientific community and Soviet citizens at large but also the rest of us.

What follows is a recipe for Mr Andropov's inquiry into Soviet science drawn up on the gloomy but realistic assumption that he will have neither the opportunity nor the inclination to follow radical policies on the questions that most sharply divide the Soviet Union from the rest of the world, the important issues of personal liberty within the Soviet state. It is, of course, offensive that Sakharov is still exiled in Gork'ii and that Orlov and the rest are still imprisoned. It is also the equivalent of tying one arm of Soviet science behind its back that the freedom of Soviet scientists to travel abroad is regarded not as an intellectual necessity but as a reward for loyalty, distinction and longevity. (Obituaries in *Pravda* regularly list a deceased's visits to conferences overseas among the other honours that may have been showered on him.) But nobody should expect that these practices can be changed overnight. The supposition that the state can order the lives of ordinary citizens is a hangover from the feudal doctrine that citizens are literally servants of the state. To acknowledge this is not to condone illiberality but merely to acknowledge that even the most enlightened Andropov would have problems on his hands.

Education

Andropov must first pay attention to the educational system, in many ways the best in the world but also the most cumbersome. Primary and secondary education are universal and state-supported. In the decades since the Second World War, Soviet schools have been acclaimed on several counts, not least for the attention that they pay to the teaching of mathematics, science and technology. In the post-Sputnik years, when curriculum developers in the West were looking for ways of improving the practice of teaching school, they found a great deal of help in earlier Soviet innovations. Yet in the Soviet Union as elsewhere, the overriding difficulties are not those of providing buildings or even schools but of recruiting able teachers. The device of segregating pre-teen students with obvious ability into special schools has demographic advantages but runs the risk, especially with the increasing social stratification of Soviet society, that, as in capitalist Britain, the best teachers will be found teaching the sons and daughters of the middle classes. National olympiads in mathematics and the like, coupled with the conviction of village peasants that a bright young person winning a place in some distant special school is a feather in the local community's cap goes some way to redress the balance, but not nearly far enough. Teaching less, for less time, would be a more equitable but also a more beneficial strategy.

American contributors please note

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Specialization

Specialization is a more serious problem, especially in tertiary education. Seriously-minded people embarking on a university career cannot even briefly enjoy the sense that the world will soon become their oyster. First degrees take far too long — four or five years is about par for the course. Thereafter, a person might hope to become a *Candidate* in his early thirties and, provided his work is not inconstant, a *Doctor* (of science, or of something else) early in his or her forties. The academic ladder is, as it seems from outside, a device for making sure that sparks of creativity are snuffed out — and for reinforcing respect for the gerontocracy. In the circumstances, the wonder is not that Soviet science tends to be derivative but that so many people have survived the system. Andropov could do worse than begin by asking for an explanation of why the system should be as it is. And why it should not be different.

The organization of Soviet laboratories is another problem. Contrary to naive supposition in the West, Soviet public administration suffers not from being excessively authoritarian but from too meticulous a division of responsibility. Even in the Red Army, military commanders are coupled with political bosses (of whom Mr Brezhnev was once one). Soviet research laboratories universally have two heads, one a scientist, one a commissar. The obvious consequence, that laboratory directors are usually less adventurous then they would be elsewhere, is written in the unglamorous record of recent decades. Paradoxically, this same system seems to allow wild eccentricity occasionally to flourish. T.D. Lysenko's influence in the 1950s would not have been as disastrous if there had not been a succession of laboratory commissars to vouch for his ideological correctness. Andropov should tackle the problem of how the state laboratories are run, and urgently.

Andropov should also worry about the Soviet Academy of Sciences, whose president, A.P. Aleksandrov, was given a place of honour at Monday's funeral orations (after the Minister of Defence, before the ritual worker). Aleksandrov was also a member of the committee that planned the funeral, but inevitably so. The trouble, however, with the academy is that its formal

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autonomy (academicians elect each other) conflicts with its function as an instrument of the state, with responsibility for administering public laboratories and, within the past year, committed to even more direct responsibility for running industrial research and development. The most obvious defect of this arrangement is that it is too reasonable, snuffing out the inevitable tension there must be between those with an interest in discovery (which is the basis of future industry) and those with an interest in current production.

The problem of the academy, unfortunately for Andropov, is not the last that calls for his attention. As in Britain, but more scandalously, there is too great a separation between civil and defence research. Only in the past few years has the Soviet space programme begun to make a bridge between the strictly secret activities of the army of defence researchers and those who serve the academy's institutes, the universities and the generality of public service. Socially and in economic terms, the relatively lowly status of the Academy of Medical Science (whose president N.N. Blotkin spoke after Aleksandrov at the funeral orations) may be unimportant, but the neglect of the Academy of Agricultural Science (whose president did not speak at all) cannot be irrelevant to Andropov's problems. Is it not time (and would it not be politically convenient) that Soviet citizens should for once be fed decently? Mr Andropov might, without disadvantage, ask the academy whose president was so much honoured on Monday why agricultural research has been so foolishly neglected. He may discover that he, like other comparable leaders, needs a mechanism by which loyal but free-minded scientists can tell him how resources should best be spent.

Unsolicited advice such as this is never welcome and often goes unread, not least by those to whom it is addressed. Others may ask why advice intended to be helpful (but likely to be overlooked) is proferred at all, in the present climate of East-West relations. There are two simple answers, which the West too often overlook. First, if the struggle (an Eastern word) between East and West is ideological, and if nuclear warfare can be avoided, engagement is preferable to detachment. Second, as both sides are fond of saying, science is international. So must not even the West be deprived by the diseconomies of Soviet science? From Artsimovich to Zeld'ovich, the West has profited from those the Soviet system has allowed to flourish: how much has it lost because so many others have been stifled?

West Germany's millstone

The new West German government seems ill prepared to tackle the problems of its universities.

The long-standing legend of the free-floating German student, moving as the fancy takes him from one seat of learning to another, is appealing but no longer a joke. The West German academic system is at serious risk of being crippled by its students, not merely by the huge numbers of them but by their disinclination to distribute themselves among West German universities according to the distribution of qualified teachers. The result is that some places are overwhelmed with students and that others are half empty. In the circumstances, the vision among academic administrators that the impending decline in the teenage population will bring relief is almost certainly an illusion; more probably, the crowded universities will be just as crowded as at present, and the others even emptier.

Everybody knows that freedom of access to higher education is deeply valued in West Germany. It is also understandable that the Social Democrat government, which helped to institutionalize the doctrine of free access with a sequence of legislation going back to 1969, could hardly have set about devising some scheme for telling would-be students where they should study. Even the introduction of the *numerus clausus* in fields such as medicine was regarded by many of its supporters as a betrayal. The new government is not bound by earlier promises, but seems no more willing to make a fight for commonsense. Will it be different after the March elections?

Ambition running cold?

Last week's meeting of the European Science Foundation was a needless disappointment.

The European Science Foundation is a remarkable organization, not a consortium of governments but an organization of publicly supported research agencies and learned academies which have agreed to contribute small (for them) sums of money to common good causes. At the outset, in 1974, there was nevertheless some reason for hoping that this shoestring organization would so command the loyalty of its customers (mostly working scientists), the fidelity of its members and the respect of their sponsors that it would have a chance of growing into an amalgam of a European academy and a grant-making organization. For the time being, however, that prospect had become too distant for comfort and good sense. Last week's general assembly (see page 207) was a poor augury for the future. The foundation has always been careful not to boast about its potential for the future, saying publicly that it had no ambitions to be more than a marginal institution. The message was appealing when it could be disbelieved. What has happened is that the foundation has begun behaving as if it were indeed a marginal institution. Why has this happened?

The temptation is to suppose that the shortage of money that now afflicts the members of the foundation has made them sour, but that is only half the truth. Domestic budgets are an excuse for parsimony, but not the cause of it. The underlying trouble, and the mainspring of the sourness apparently running through last week's meeting in Strasbourg is that the member organizations have become more inward-looking than they were nearly a decade ago. If that were not the case, they would surely have acknowledged the logic of their own laments at the prospect that good causes such as the fellowships and summer-school programme in brain and behaviour research will founder unless somebody finds another sponsor within two years, and have recognized that they had best dig deeper into their own pockets. If such a programme is acknowledged to be worthwhile, and if those who now contribute towards its cost through the European Science Foundation are to be believed when they say that they would contribute to the same programme under a different umbrella, why can they not see that they would be as well served, and the foundation in the process strengthened, if they made Strasbourg the channel for their largesse? The snag seems to be that the mood has changed. A decade ago, people were looking for pan-European institutions. Now they are more conscious of the respect that they can earn on the international scene by being themselves the sources of the small sums of money flowing into international enterprises.

The foundation should nevertheless not despair. Some of its difficulties are procedural — its own cumbersome ways of conducting business, for example, and constitutional problems among its member states, such as that which prevents Austria from contributing honest schillings to other people's running costs. The first of these could and should be removed by means such as those accepted last week, the second suggests that a treaty between governments might after all be preferable to the present clubbiness. The foundation should also seize on the opportunities that now abound for providing the scientific community with an understanding of the changes that are taking place within it. Even relatively simple questions, such as the amounts of money spent on different kinds of science in different countries (once tackled systematically by the Organization for Economic Cooperation and Development) are now neglected. The changing demography of the academic profession throughout Europe, practical questions of the employment of scientists in different states or of the proper relationship between academic research laboratories and industry (the catchword "technology-transfer" applies) could with advantage be taken up. If European organizations are for the time being short of European spirit, the foundation had better find a bread-and-butter role to keep itself alive until the spirit waxes again.

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Stanford's hope for heavy boson

Linear machine may give an edge on CERN

Washington

Physicists at the Stanford Linear Accelerator Center are keeping their fingers crossed during the next few weeks while the Office of Management and Budget (OMB) decides on their $44 million request to begin construction of the Stanford Linear Collider (SLC) in fiscal year 1984. Approval would put SLC in the running with CERN's large electron–positron storage ring (LEP) to be the first machine to produce the roughly 90 GeV electron–positron collisions needed to generate the elusive Z° particle.

"If we get that funding," says Burton Richter, director of the SLC project, "we would be ready to turn on in October 1986". The $44 million would be the first instalment of a total of $110 million required for construction on a three-year schedule. LEP is scheduled for completion in late 1987.

Richter admits that the possibility of beating CERN to the punch has helped fuel enthusiasm for SLC. That enthusiasm seems to carry through the physics community and the Department of Energy's high-energy physics office, a broad base of support that may help the proposal over the twin hurdles of OMB and Congress. OMB's decision will not be made until December, and will not be made public until the President's 1984 budget is released at the end of January. At present, though, indications are that SLC may get the go-ahead. Earlier this year, the Energy Department's High Energy Physics Advisory Panel (HEPAP) strongly endorsed the project, recommending a 1984 start.

William Wallenmeyer, who directs the Energy Department's high-energy physics programme, points out that SLC has already rated "rather high priority"; the 1983 budget provides about $10 million for research and development in connection with the project.

But in its closed-door deliberations, OMB may find several reasons to delay construction. Chief among them is that the physics aspect of SLC — namely the ability to study the Z° — is only a part of the rationale behind the project. Wallenmeyer says the "primary justification" is that SLC would allow a new concept in accelerator design, called the "colliding linac", to be tested. This new breed may prove to be a less expensive alternative to the circular accelerators.

Thus a modest delay in SLC would not harm this primary goal. SLC would be built by adding a loop at the end of Stanford's existing linear electron accelerator. Electrons and positrons would be accelerated down the linear stretch; the electrons would then follow one branch of the loop, positrons the other, and they would collide at the far side of the loop. Energies of up to 50 GeV per beam (100 GeV in the centre of mass in the collision) are anticipated.

Another possible counter to Stanford's push for a 1984 start and a quick three-year construction schedule is that the physics that can actually be done on SLC may in fact be rather limited. George Trilling of Lawrence Berkeley Laboratory chaired a HEPAP subpanel that last January recommended that SLC go ahead in 1984 even under a "low-budget" option — with the savings to come from a cut-back in Brookhaven's ISABELLE accelerator project; yet Trilling says that SLC is "not anywhere on the scale of LEP or ISABELLE; it's of a more limited scope". LEP, he says, will be of general use to high-energy physicists, while the physics on SLC will be limited to the Z° work. And Wallenmeyer points out that the actual discovery of the Z° is likely to come from an existing machine at CERN, which collides proton and antiproton beams. "The purpose of LEP or SLC would be to pursue the resultant physics that comes out of that", Wallenmeyer says. The electron–positron collisions in these machines make the interpretation of decay products much simpler.

None of this, however, discourages Richter in his role as director and chief proponent of SLC. Although SLC will have a single detector as opposed to LEP's four, he says, "SLC has as much physics potential as LEP." Furthermore, he says, SLC will provide a smaller beam (1.4 μm radius compared with LEP's 500 × 50 μm cross-section), making the decay of short-lived particles easier to study; SLC will also be able to generate longitudinally-polarized beams, a boon in attaining high precision in weak-interaction experiments.

Richter also discounts the significance of LEP's higher energy capability. In its current design configuration, LEP will produce energies up to 80 GeV per beam; going beyond that, Richter says, would require a new technology as yet untested. Richter says that once the Z° resonance is reached at what is predicted to be 94 GeV (47 GeV per beam), there is no interesting physics until about 200 GeV (100 GeV per beam), when pairs of charged W^+/W^- particles would begin to be produced in significant quantity.

For all of the build-up, the race between LEP and SLC may be determined in the starting gate if OMB decides to postpone construction of SLC. But if SLC gets the go-ahead for a 1984 start and a three-year construction schedule, it may be a race down to the wire. **Stephen Budiansky**

CERN unafraid of Stanford

Undismayed by the news from Stanford (see accompanying story), the European centre for nuclear research (CERN) is still hoping that it will find the heavy boson in the next few months.

Over the past 4 weeks, CERN's system for colliding protons and antiprotons with each other has been working much better than before. "We are now really in business", said CERN director Professor Herwig Schopper last week. The collisions should produce both the charged intermediate vector bosons, the W^+ and W^-, and the neutral one, the Z°. The collider is working well enough to produce "a few" bosons before Christmas, although it will be difficult to distinguish them from the many other particles produced in the high-energy collisions. The interest in the particles is that their existence is a clear prediction of presently widely accepted unified field theories, so that their detection will provide a critical test of the theories.

Meanwhile, contracts are now being prepared for around a third of the components for the construction of the large electron–positron collider, LEP, which is CERN's next major project. LEP is designed to give the clearest possible investigation of intermediate boson and related physics. The first contracts to be agreed have been for a major section of the 27-km tunnel, for the production of 3.6 million magnet laminations and for steel and concrete cores for the magnets. They have gone to the lowest bidders, which have been consortia involving Italian, French, German, Swiss and Austrian companies. The prices have been roughly as planned, and certainly not low enough, says Schopper, to bring forward the completion date of late 1989.

Construction will not, however, begin until the French authorities give their environmental seal of approval. Two-thirds of LEP lies in France, and one affected village, Echevenex, has made a particularly strong protest (see *Nature* 30 September, p.385). The local review procedure is now over, and CERN must now make its own representations to the committee. CERN has two possible new LEP designs for the Echevenex region. The committee will submit its recommendation to the French government which is expected to come to a decision early next year. **Robert Walgate**

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European/Soviet collaboration

Doubts surface

The Committee of the Council of the European Laboratory for Particle Physics (CERN) has failed to reach an agreement on the proposed renewal of its collaboration with the Soviet Union. The Soviet side is anxious for this collaboration to be extended, particularly in view of the construction of CERN's new electron-positron collider (LEP).

The lack of agreement among the committee about the renewal reflected the continuing concern about human rights in the Soviet Union, in particular the continuing repression of such "eminent scientists" as Dr Yurii Orlov and Academician Andrei Sakharov. Since 1978, when Orlov received a 12-year sentence, CERN has taken a special interest in his fate.

The CERN delegates reflect the differing views in the West on how far the human rights issue should be linked to decisions on scientific and trade cooperation. The United Kingdom delegate, for example, expressed a belief in the importance of collaboration in science, saying that Soviet participation in LEP experiments "would be of concrete benefit to the international scientific community", although admitting to concern at the "present record of the Soviet Union in relation to human rights".

The Italian delegation, however, felt that such a sensitive issue should be settled only with the approval of the wider scientific community. They urged, therefore, that a new cooperation protocol be drawn up for ratification by the CERN Council, whose proceedings are public, rather than simply by the committee in closed session.

The machinations of the CERN committee were undoubtedly influenced by the imminence of the Madrid Review Conference of the Helsinki Act, which reopened on 10 November. The CERN Orlov Committee was a co-sponsor, with the Comités Scientifiques Françaises and "Scientists for Sakharov, Orlov and Shcharanskii" (United States), of a special press conference in Madrid, designed to draw attention to the plight of their Soviet colleagues.

The death of Leonid Brezhnev, and the accession to the post of General Secretary of the Communist Party of the Soviet Union of Yurii Andropov will inevitably evoke considerable rethinking of East-West scientific exchange. Although in his funeral oration on Monday, Mr Andropov expressed a commitment to detente and a willingness to cooperate with any country which genuinely sought cooperation, he also pledged support to the "strengthening" of socialist government at home and abroad, which could well lead to actions unacceptable to human rights lobbies in the West. **Vera Rich**

French science research

Papon explains his plans

The Centre National de la Recherche Scientifique (CNRS), the research council which dominates basic science in France, seems to have set off on the right foot with its new director-general, Professor Pierre Papon, who came to the post hot-foot from a political position at the ministry of research and industry in October.

Initially, some feared that Papon's close links with the ministry would increase political influence over fundamental research policy — but his first statement of position to the CNRS council a few days ago confirmed the continuity of his thinking with that of his predecessor, Professor Jean-Jacques Payan, and even hinted that CNRS may influence the government, rather than the reverse.

Papon explained that he had four main objectives to achieve: to create "a new dynamics" in CNRS science; to "open" CNRS to cultural, economic and social needs, and towards the international community and the Third World; to "democratize" decision-making; and to make CNRS "one of the main partners" of the government in the whole process of national science policy-making.

By "new dynamics" Papon means to create new areas of research and to stimulate others — such as general microbiology, or the neurosciences — which are not considered to be at a sufficiently high level in France. Also French science is often strictly compartmentalized, and Papon intends to help break these barriers and induce the movement of ideas and people by setting up new interdisciplinary programmes, such as the programmes on drugs (PIRMED) and materials (PIRMAT).

Papon also wants to loosen the distinction between pure and applied research — although, he insists, "of course CNRS must remain 80 per cent basic". His guiding light is Jean-Frédéric Joliot, the Nobel-laureate radiochemist who was the first director of CNRS in 1944–45. "Joliot thought of applications of radioactivity in the 1930s, when Rutherford was dismissing it as a joke" said Papon. Joliot, although a basic scientist, took out early patents on a primitive radioactive pile. He demonstrated that the pursuit of truth and profit are not incompatible.

As for "opening" CNRS to the outside world, Papon will create two new directorates, one to pursue the application of CNRS results in industry, and one to deal with information, publicity and international relations. Papon, a socialist, stresses his own interest in improving CNRS links with developing countries. But his policy here will be guided by French foreign policy.

The third line of Papon's policy, "democratization", he interprets as "complete transparency": decisions will still be taken by the directorate, but in the open, after wide debate.

The fourth line, making CNRS a partner in government policy-making, means essentially that Papon, with his strong political links and experience, will not hesitate to make positive science political proposals to the minister of research and industry — and other ministers. There will be no waiting meekly for the directives to come from above.

On other issues, Papon is convinced of the value of the 12-year-rule (see *Nature* 14 October, p.569, and 21 October, p.670) by which laboratory directors will have to resign their posts after 12 years in the job; and believes that Dr Maurice Godelier, an anthropologist who a year ago was bitterly opposed as potential director for the social sciences at CNRS, is now broadly acceptable to the social science community "as far as I can tell". Godelier's report on the social sciences in France (see *Nature* 28 October, p.771) is "a masterpiece", according to Papon, and generally agreed to be a good assessment. The appointment of a director for social science and humanities should be made in a few weeks, and although Papon would be happy to work with Godelier he would also accept "any open-minded person" in the job.

On the 12-year-rule, Papon believes it helps to know you have a finite time in which something concrete must be achieved. The only problem is with the smaller groups, set up around one scientist whose ability has been the *raison d'être* of the team. But the rule, as later qualified by the research and industry minister, leaves room for manoeuvre. **Robert Walgate**

Good news for BAS

The Falklands War seems to have worked to the advantage of the British Antarctic Survey. The Department of Education and Science announced last week that it is to allocate an extra £4 million in 1983–84 in addition to the normal allocation expected from the Natural Environment Research Council (NERC). After a long period of tight budgets, this appears to be the beginning of an expanded commitment by the British towards Antarctic research. NERC's allocation to the survey for this year, £7.8 million, is already significantly more than last year's £5.6 million.

No reason was given for last week's announcement, and it is not yet known how the extra money will be spent. The Falkland Islands are used as a refuelling and supply base for Antarctic survey support ships. At present there is a numerically superior Argentinian presence in the Antarctic Peninsula, with 158 Argentines (132 of them military personnel) and 58 British survey scientists. David Millar

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European Science Foundation

Living with level funding

Strasbourg

This year's general assembly of the European Science Foundation (ESF), at the European Parliament on 9–10 November, was curiously disappointing. For almost the first time since its creation in 1974, the foundation spent as much time deciding how and when to bring good projects to an end as on the problems of how best to launch new enterprises.

Part of the trouble is that the foundation seems to have been infected with the present preoccupation of its members — all of them research organizations in the member states (which include Jugoslavia, Turkey and Israel as well as countries in Western Europe and Scandinavia) — the problem of living within a constant budget. In this spirit, the assembly spent much of its energy this year wrestling with the question whether one of its first and most successful programmes, the European Training Programme in Brain and Behaviour Research, should be pushed towards independence one, two or three years hence. But the assembly's consideration of new projects has also been strangely flat and uncritical.

The foundation is for practical purposes a club whose members are publicly supported grant-making agencies in the member states. Each national group pays a collective subscription determined by its home state's Gross National Product, but may then contribute voluntarily to the several "additional activities" that make up the foundation's research programme.

In such circumstances, it is hard to tell how large is the effort subsumed under the umbrella of ESF. This year, for example, central expenditure will amount to FF6.8 million (£0.5 million), but cash contributions for additional activities will amount to FF5.7 million and there seems to be a gentlemen's agreement that overhead costs should be concealed wherever possible. The proposal that central expenditure next year should rise to FF8.1 million — rather less than 0.05 per cent of the budgets of its members — was accepted without a trace of dissent, no doubt in gratitude for the foundation's implicit undertaking that from now on it will take on new projects only when it has discarded others.

The counterweight for the proposed abandonment of the brain and behaviour programme is the European Geotraverse project, a plan to mount a series of deep seismic soundings along a north–south band running from northern Norway to Tunisia, together with coordinated geological, geochemical, geomagnetic and magneto-telluric measurements (see *Nature* 29 July, p.413). The project was enthusiastically endorsed by the assembly, or at least by those of its members whose national boundaries lie near to the traverse.

Enthusiasm does not however mean money. The traverse project will require 14 million Swiss francs (£3.75 million) for central funds, while the planners (led by Dr P. Fricker, secretary-general of the Swiss National Science Foundation) hopes that a further FF8 million will be spent by member states on regional studies. In the event, roughly half the countries concerned were able to commit themselves, in a genteel version of a political fund-raising affair, to contribute to the costs of coordinating the enterprise.

In future, the foundation hopes to have a better mechanism for recruiting contributions towards such additional activities, the assembly having adopted at this meeting a set of proposals negotiated by a group under Dr Fricker that will allow interested members' budget contributions to be negotiated while projects are being developed. As things are, members have no cause to dissent in public from proposals they consider to be half-baked, knowing that silent assent need cost them nothing.

For the rest, the assembly reappointed Professor Herbert Curien as president for a further three-year term, heard Sir Hermann Bondi plead for a better understanding and appraisal of environmental problems, agreed that something should be done to improve legislation covering archaeological sites, agreed that similar laws are needed to secure meteorites for science, applauded those of its members seeking to persuade some European government to play host to a European source of synchrotron radiation and approved a plan to hold an interdisciplinary meeting on the criteria for progress in science in whose specification the word "discovery" does not appear. ●

European space research

Backing away from Ariane?

European astronomers, increasingly concerned about the delays in the Ariane rocket programme, are pleading that the European Space Agency (ESA) should consider launching their next X-ray satellite, called EXOSAT, on a Thor-Delta rocket. A decision to switch launchers at this stage would embarrass (but possibly relieve) Arianespace and cause contractual problems (but probably decrease the cost). According to one senior X-ray astronomer, however, the case for switching launchers is now undeniable on scientific grounds.

The three-stage Ariane rocket has failed, for different reasons, on two out of five of its launches. A committee of inquiry has determined that the failure on the last launch was due to problems with lubrication and design tolerances in the rocket's turbopumps. These are being modified before the next launch in April. On present plans EXOSAT would be launched on the following Ariane in the middle of next year.

EXOSAT, the only X-ray astronomy satellite expected to fly for several years, represents a significant investment over the past few years by European astronomers. Several aspects of its design reflect the recent rapid development of X-ray detectors.

The satellite was originally designed to be launched on a Thor-Delta rocket, and the subsequent decision to change to Ariane had a strong political (chauvinistic) element. Accordingly, only minor structural modification would be necessary to go back to a Thor-Delta launcher, which has an enviable record of successful launching.

Now British, German and Dutch astronomers have written to ESA asking that EXOSAT be an urgent item for discussion at the December meeting of the Science Programme Committee and requesting that "all possibilities be considered". Any recommendation made by that committee would have to be ratified by the council of ESA, which would normally place much weight on the programme committee's recommendations.

A Thor-Delta would cost about 10 million accounting units (AU) — about £5.4 million at current prices — less than Ariane but ESA has already paid Arianespace 30 million AU for the EXOSAT launch. Arianespace might, however, be relieved not to have to launch EXOSAT. First, it is under pressure from commercial users to launch their satellites on schedule. Second, the EXOSAT launch would require a hitherto untested fourth stage to be added to Ariane, leading to the increased possibility of another failure.

There are other technical reasons why X-ray astronomers would push hard for a switch. Thus it is possible that a Thor-Delta could be ready in time to launch EXOSAT at the end of the current window. (EXOSAT's orbit is highly elliptical, permitting more extended observations of variable sources and more continuous contact with ground tracking stations than with previous X-ray satellites. The "windows" are those periods in the year during which a launch would provide the appropriate combination of solar cell illumination and the correct location of the orbital apogee in the sky.) With Ariane, this window ends in January, and another launch would not be possible before the middle of next year. The use of a Thor-Rocket, obviating the need of Ariane's transfer orbit, would extend the window to February, giving the possibility of an "early" launch and the avoidance of six months' degeneration of X-ray detectors stored in laboratories. **Philip Campbell**

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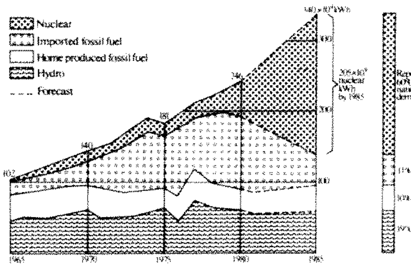
French nuclear power

Testing time for reactors

The problem with French nuclear power stations is that three-quarters of them are less than three years old, and suffering from teething problems, according to Electricité de France (EDF), which owns and runs them.

What is not clear is whether this should be a comforting or a disquieting thought. French nuclear power production is supposed to be increasing, but in September EDF produced only 6,700 GWh of electrical energy, compared with 8,200 GWh a few months previously. The net availability — the proportion of energy delivered to nominal capacity — fell to below 50 per cent in the summer, compared with a typical industry target of 70 per cent and a world average for pressurized water reactors (PWRs) (according to EDF) of 60 per cent. But, says EDF, PWRs more than three years old had the magic 70 per cent availability. It was the young ones — an independent design series of 20 or 50 — that were in trouble.

The accompanying figure reveals how much of the French nuclear power programme is indeed young, coming on line during 1980–85. This is literally a testing time for French nuclear power. Of the 22 reactors now connected to the grid, only 6 were connected before 1980. Seven were connected that year, seven in 1981, and two have been connected this year. However, another 30 are under construction, due to come on line during 1983–89.



Power consumption in France

According to EDF, the problems amount to a combination of technical teething troubles and government safety regulations. There are three issues: a concentration of seven four-month shut-downs for obligatory inspections, after 18 months' operation, compared with four such shut-downs in 1981 and two planned for 1983; and two problems with faulty materials in the younger reactors.

One of these problems is outside the nuclear part of the reactor, in the steam "drier-superheater". This halted two reactors, Saint-Laurent B1 and B2, for a considerable period, but the problem is now thought to have been solved for these reactors and others in their production series.

The more awkward problem is the stress-corrosion cracking of inconel clips holding guide tubes for the control rods within the reactor vessel. The problem was first noted in Japan in 1979, and since then the clips have undergone redesign in French reactors beginning construction. But most reactors coming on line in the past three years have the old clips, and there have been failures and shut-downs this year at Fessenheim 1, Bugey 2 and 4 and Gravelines 1. The clips are to be replaced on a series of 20 reactors, at a net cost, according to EDF, of about FF 1,300 million (£110 million).

But is this the end of the "teething" troubles — or will there be more to come, when the problem, like the clips and the drier-superheaters, will be multiplied through a long production series leading to high replacement costs, and a degree of insecurity in future electricity supply, which is planned to reach 60 per cent nuclear by 1985?

However, this can be taken as an argument for building more nuclear rectors, not less, to provide a cushion of overcapacity, and it will no doubt be taken that way at the ministry of research and industry in the forthcoming argument over coal and nuclear power and falling energy demand forecasts (see *Nature* 11 November, p.100). In Britain, too, where the Central Electricity Generating Board is planning its first PWR at Sizewell, Suffolk, the French problems are seen in a positive light — as useful design lessons. **Robert Walgate**

US defence

Townes in dissent on MX siting

Washington

Controversy continues about how and where the new MX missiles should be based. President Reagan is committed to give Congress a plan for basing the MX (short for "missile experimental") by 1 December and the Secretary of Defense, Mr Caspar Weinberger, has done his part by transmitting his recommendations to the White House, apparently on 1 November. But now his most prominent scientific adviser on the issue, Dr Charles Townes of the University of California at Berkeley, has taken the unusual step of expressing his personal doubts about the now-favourite plan — and the contents of his letter have been promptly leaked in the US press.

The question of how to deploy MX has been a long-standing conundrum. Out of about 30 alternative plans suggested by the military, the Carter Administration selected that called "Racetrack" in which missiles would have been moved from one launching point to another by means of underground tunnels. But by the time the plan was put forward, so many doubts had arisen that Congress never approved it.

The same fate may yet await the Reagan Administration, which has already failed to persuade Congress to authorize funds for deployment — without specifying how the missiles would be deployed. Townes was appointed head of a panel of scientists and defence experts to re-examine the MX problem when Mr Weinberger took office. The scheme now most favoured by the US Air Force and, it is rumoured, by Mr Weinberger is called "Dense Pack". MX missiles would be placed in fixed and hardened canisters in the ground so close together that incoming warheads would interfere with each other, the blast and other consequences of a nuclear explosion interfering with later arrivals.

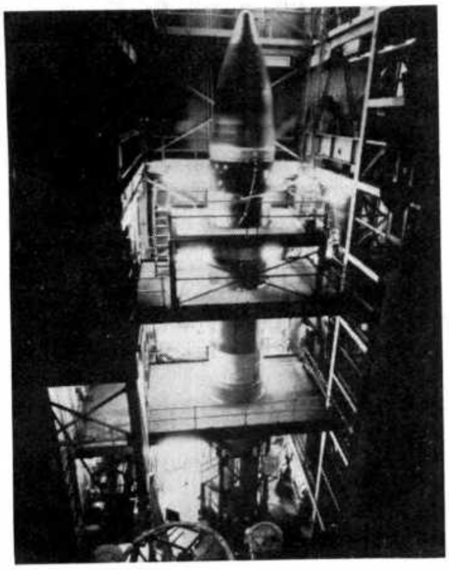
The Air Force now says that 100 MX missiles separated by distances of about 2,000 feet would be close enough to ensure interference (called "fratricide" in the new jargon). Hitherto, the Air Force has discarded Dense Pack on the grounds that missiles not destroyed in an attack would be unable to fly out in retaliation. The new development is that the Air Force says that missile silos can be hardened so that their missiles will survive even if over-pressures amount to 5,000 lb per square inch.

The nub of Townes's argument is that this "super-hardening" may not be feasible, that there are no clear limits on the time that would be needed for such a development or of its cost and that the Soviet Union might find a counter to Dense Pack sooner than the Air Force could install it.

Military considerations apart, Dense Pack has the political advantage that it would occupy an area of only 10–15 square miles. One of the objections to Racetrack from ranchers and environmentalists in western states was that huge tracts of land would be required.

"FRATRICIDE—
HOMICIDE—
SUICIDE—IT'S
ALL THE SAME
TO ME."

The latest version of Dense Pack advocated by the Air Force is that the 100 MX missiles would be sited in a long thin column aligned north to south. This has the theoretical advantage that the optimum pattern of an attack on the missile field from the Soviet Union, called a "walk attack", can be predicted: the most southerly rows of missiles would have to be attacked first and succeeding warheads would have to be aimed at suitable intervals at rows of missile silos sited further north. On this argument, a pattern of attack moving from north to south would be ineffective because later warheads would have to survive the atmospheric disturbance caused by the first nuclear explosion.



It is not clear what the consequences will be for Dr Townes now that his strong expression of personal doubt about the government plan has leaked into the press. Scientific advisers have run into trouble on such grounds on many previous occasions. But Dr Townes is much respected within the present Administration and his opinion may even help the President to reach a wiser decision by the beginning of next month. **Deborah Shapley**

Yes to toxin cloning

Washington

The Recombinant DNA Advisory Committee (RAC) approved two controversial toxin-cloning experiments at its 25 October meeting, but ordered that they be carried out under P4 containment, the most stringent safety precaution available.

Dr John Murphy of Harvard Medical School, who in July 1981 received permission to clone the diphtheria toxin gene in *Escherichia coli* K-12, will now be allowed to extend those experiments to linking the toxic gene with the gene that codes for melanocyte stimulating hormone. The aim is to generate a hybrid toxin molecule that selectively homes in on melanoma cells.

The experiments will have to be performed at the National Cancer Institute's laboratory at Frederick, Maryland, which is the only P4 facility in the country. **Stephen Budiansky**

German research and technology

Waiting for the next election

Bonn

Dr Heinz Riesenhuber, appointed last month as West German federal minister for research and technology, seems to have made a good first impression. But it is also clear that between now and the promised general election next March, there will be time only for some modest if urgent housekeeping, and for planning how to put flesh on the bones of the promises of the past few weeks.

The federal ministry for research and technology has two immediate tasks — to win agreement on a budget for next year (and on a supplementary budget for 1982) within the caretaker government and with the parliament; and to put the two big prototype nuclear reactor projects on a sound financial basis. Both issues must be settled before the year is out, for thereafter all contentious issues will be overtaken by preparations for the election.

The budgetary and reactor issues are closely linked, for the reactor construction costs are a large part of the federal ministry's budget. There seems a good chance that the parliament (Bundestag and Bundesrat) will fall in with the previous government's proposal that the ministry should have a supplementary subvention of DM500 million (£116 million) with which, for practical purposes, to liquidate bank loans taken out by participating electricity utilities to keep construction going. (The propriety of this device could nevertheless become a political issue at the election.) If the Christian Democrat government is re-elected, Dr Riesenhuber's belief that both reactors should be completed will nevertheless become a simplifying touchstone for future policy: although, as one high official emphasized last week, the government will not support the projects "at any price". But the new government seems to understand that new technology is never more expensive than when it is postponed.

The implications of Dr Riesenhuber's first-flush declaration that research and technology must share in the new government's commitments to a six per cent reduction of spending, but that "basic research" must at the same time be protected, are harder to discern. Some officials hope to find some relief from the strictest possible application of the six per cent rule in the *caveat* that it may not apply when the consequences would be legally invalid. Most of the ministry's projects entail agreements with constitutionally autonomous Länder governments for example, and may therefore be exempt. The budget for 1983 now being canvassed in the Bundestag, at just under DM7,000 million, is a reduction of DM300 million on the original budget for 1982 — the first reduction since the ministry's creation in 1971. But it seems to be an article of faith that when economies are decided, they will not affect basic research.

Dr Riesenhuber's other memorable declaration, that the large national research laboratories should have a closer relationship with industry and that direct support for research and development should progressively be replaced by "indirect support", is for the time being equally uncertain in its implications. The minister's conviction that civil servants cannot adequately supervise the more than 6,000 direct-support projects now on his department's books is readily accepted, but nobody has a clear idea of what indirect support consists of. The notion that the federal government might subsidize the salaries of qualified scientists and engineers working for small and medium-sized companies is one being canvassed. By the general election, Dr Riesenhuber may have other cards up his sleeve. Meanwhile, he is committed to the award of a prize of DM30,000 at some point during 1983 to somebody working in a public laboratory making the most outstanding contribution to industry.

No doubt in the hope of avoiding similar problems with thermonuclear fusion, the federal ministry of research is planning to peg its total expenditure on fusion, principally at the Max Planck Institute at Garshing and the JET laboratory at Culham in the United Kingdom, to about DM200 million a year. It has been decided that Garshing will be concerned only with plasma physics machines, and that the nuclear research establishment at Karlsruhe will assume responsibility for any experiments in which thermonuclear fuel is consumed to produce radioactive materials.

Elsewhere, the prospect that a change of government may stimulate other changes seems now, as ever, frustrated by the constitution. Nobody thinks it feasible that the excess numbers of students at the most popular universities could be limited by some selection process, or even arbitrarily; the Länder would not stand for that. The federal ministry of education is nevertheless hoping to help cure the enforced immobility in academic life in the past few years with a proposal to spend DM50 million a year on the appointment of up to 1,000 newly graduated PhDs to one- or two-year posts in universities. This development, valuable in itself, is also a test of the federal government's undivided constitutional responsibility for research. The bill now before the Bundestag does not depend on the compliance of the Länder governments.

The education ministry, following the doctrine that basic research should be protected, has also managed to increase the budget of the Deutsche Forschungsgemeinschaft for next year by four per cent. ●

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European Molecular Biology Laboratory

Crucial decisions ahead

Heidelberg

This month is crucial for the European Molecular Biology Laboratory, supported since 1974 by eleven European governmental research agencies, a subset of the seventeen members of the European Molecular Biology Organization. Next week the governing council of the laboratory will decide whether it can match with deutschmarks its acceptance of a new scientific programme at its meeting last June. The director of the laboratory since 1 April, Dr Lennart Philipson, is dismayed by suggestions such as that in the report of the British Advisory Board for the Research Councils (see *Nature* 4 November, p.1) that the Medical Research Council should "review" its membership of the laboratory.

The Medical Research Council refuses to comment on this recommendation. It is however known that its secretary, Sir James Gowans, paid an unofficial visit to the laboratory in September, since when there have been two visits by parties of officials to the laboratory and its two outstations at Hamburg and Grenoble.

Whatever the UK research council's attitude next week, the council meeting is certain to be uncomfortable. Philipson is asking for budget contributions of DM36.7 million (£8.5 million) for 1983, a substantial increase on the total contribution of DM 30.2 million in the present year. Philipson emphasizes, however, that a direct comparison of these figures is misleading. In past years, the laboratory has consistently failed to recruit up to its planned strength, with the result that there has usually been a cushion of unspent appropriations to help with the succeeding year's budget. During the present year, members' contributions have been augmented by more than DM6 million from this source, but for next year there will be a cushion of only DM2 million.

The perplexing question now is whether next week's council will accept that the planned increase in expenditure amounts only to 6 per cent, or whether it will take fright at being asked for a 20 per cent increase in members' contributions.

The more distant but more important question of what the laboratory is for has, however, been settled by the scientific plan agreed in June. The laboratory now accepts that there is no means by which its existence can be justified on grounds comparable with those applicable to international laboratories such as CERN in Geneva. While the synchrotron radiation and neutron diffraction facilities (at Hamburg and Grenoble respectively) are much used, especially by workers from smaller countries, they are not unique. And the prospect that the low-temperature electron microscope developed at the laboratory might be a focus for international collaboration has receded as the predicted resolution of that instrument has decreased (see *Nature* 30 September, p.386).

The new programme entails that the instrumentation division at the laboratory will become a source of new technology in molecular biology for the laboratory and member organizations. The development of the low-temperature electron microscopes will continue, but the excellent workshops will be increasingly used for building prototypes of instruments for which there is a known need.

In molecular biology proper, the laboratory is best known for its work on membrane proteins. On the basis of workshops held earlier this year, it is now planned also to concentrate on two new fields — protein–DNA interactions (as in the functioning of regulatory nuclear proteins) and differentiation (as typified by the haematopoeitic system. Dr Thomas Graf has been recruited from the nearby German Centre for Cancer Research (on a five-year rolling contract) to help develop the second line of inquiry. Meanwhile, the structural group at the laboratory will concentrate on emerging opportunities for the structural analysis of membrane proteins.

The long-term objective is thus to create a recognized centre of excellence, but also more fully to implement the laboratory's brief as a training centre. There will be one summer course next year, and a series of courses between June and September in 1984. Meanwhile, the laboratory is arranging with European universities to recruit up to ten graduate students a year.

The development of the scientific programme is the source of Philipson's immediate difficulties. The laboratory's target payroll of 267 people (excluding visitors) has never been filled, but next year's budget allows for 255 people, a substantial increase on this year. There is also to be a system of external reviews of scientific programmes at intervals of three years.

It seems to be accepted that in its new form the laboratory will be of more immediate value to member organizations too small to sustain substantial molecular biology laboratories of their own. But Philipson says that there is one sense in which Heidelberg is likely to become unique — it has been laid down that scientists on the payroll should not have external links with commercial organizations, although they may work as consultants during vacation time. ●

Industrial sponsorship

Fewer payrolled students

Industrial sponsorship schemes, under which a favoured proportion of Britain's 251,000 undergraduates receive a substantial financial boost from industry, seems to be on the decline. A better understanding of the trend is likely to come from a new research project to be conducted by the Institute of Manpower Studies (IMS) at the University of Sussex.

In some subject areas, notably electrical engineering, as many as 50 per cent of all undergraduates may be sponsored, according to Richard Pearson, head of the IMS Labour Market Studies Group and director of the study. Yet despite the obvious importance of the practice for graduate recruitment patterns in general, there is a striking absence of hard information about figures and trends. Sponsorship may mean up to an extra £800 per year to an undergraduate, and some employers, such as British Telecom, actually reward their favoured potential recruits with a salary while they are still at college. In return, there is a gentleman's agreement that the student will accept any offer of employment from the sponsoring company after graduating. Of undergraduates who started their courses in 1980, 2,000 at universities and nearly 1,000 at polytechnics found an industrial sponsor, mainly in the fields of electrical and mechanical engineering. The IMS study, which is supported by a £55,000 grant from the Leverhulme Trust, will concentrate on sponsorship by individual companies in both public and private sectors.

Most of the sponsorship schemes now operating started in the late 1970s and apply to the general area of engineering, where there was until recently a severe shortage of suitably trained graduates. Up to 50 per cent of all students taking sandwich courses (which include one or more periods of training in industry) are thought to receive some form of sponsorship. Dr Clive Purkiss, director of IMS, has drawn attention to the lack of clear information on which employers can base sponsorship decisions. Although industrial sponsorship may be regarded as being partly a public relations gesture by the sponsoring companies, it does enable them to get an inside track in their graduate recruitment programmes. It may also be the cheapest way of ensuring an adequate training in a specialist discipline, which according to one estimate may otherwise cost up to £75,000 to the age of 25.

The past two years, however, have seen a drop in the number of sponsored places available: British Telecom's total is down slightly this year to 32, and BP Ltd has just axed its scheme altogether According to Professor Ray Wild, of Brunel University's Special Engineering Programme, this is partly because companies have become more selective, preferring now to identify

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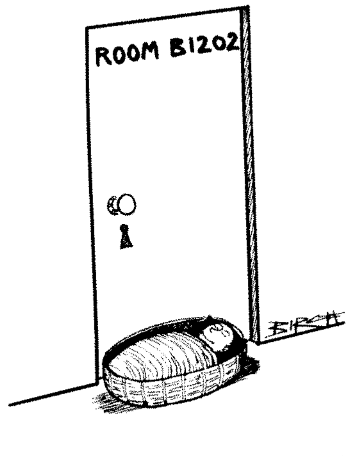
potential recruits before they start a degree course rather than to pick them out part of the way through.. The number of students on National Engineering Scholarships (financed jointly by the Department of Education and Science and the Department of Industry) has also been reduced this year, from 300 to around 100. At the same time, educational institutions are trying to increase sponsorship for their students. Some colleges actually specify industrial sponsorship as a requirement for some of their courses, as at London's Imperial College. Students are simply issued with a list of potential sponsors and told to make their own arrangements; the value of the sponsorships on offer varies considerably.

At the moment few small firms sponsor undergraduates, so major employers who run generous schemes can take their pick of the best potential engineers and managers Less well known firms have to compete against household names in the minds of school sixth-formers who have little knowledge of the industry: Brunel University, whose engineering students are all fully sponsored, is organizing a consortium of several groups of small companies to help overcome this difficulty. The IMS research should receive widespread attention, not least from the students themselves. **Tim Beardsley**

Warnock seeks evidence

The chairman of the British government's inquiry into human fertilization, Mrs Mary Warnock, has asked for all interested bodies and individuals to submit evidence before the deadline of 1 March 1983. The enquiry, due to report in 1984 has a broad brief — covering social, ethical, legal and medical aspects of developments in human fertilization including *in vitro* fertilization techniques. Evidence should be submitted to: The Secretary, Inquiry into Human Fertilization and Embryology, Room B1202, Department of Health and Social Security, Alexander Fleming House, London SE1.



UK graduate employment

No jobs for the boys (or girls)

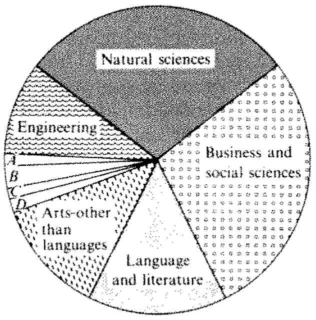
About 15 per cent of this year's crop of first degree graduates from British universities will still be looking for jobs at the end of the year. This gloomy estimate was made by Mr B.E. Steptoe, chairman of the statistics subcommittee of the Association of Graduate Careers Advisory Services; 11.3 per cent of that year's graduates had not found jobs by the end of December. Among polytechnic graduates, the figure is likely to be even worse.

The position of this year's 105,300 new graduates is, of course, made worse by the fact that they are now competing for jobs with those of the 1981 crop still unemployed. As a result, many companies that previously recruited graduates straight from college are now looking for people with some useful work experience. There are also increasing numbers of "underemployed" graduates doing jobs that previously would not have required a degree; some of these will undoubtedly find more suitable employment in time, but a large proportion are likely to remain underemployed.

The trend is described by Mr Brian Putt, director of the Central Services Unit (an advisory body to graduate careers services) as a "disastrous social iceberg". The number of graduates entering the job market will continue to rise by about 2-3 per cent each year at least until 1984, when the effects of last year's cutbacks in higher education places will become apparent.

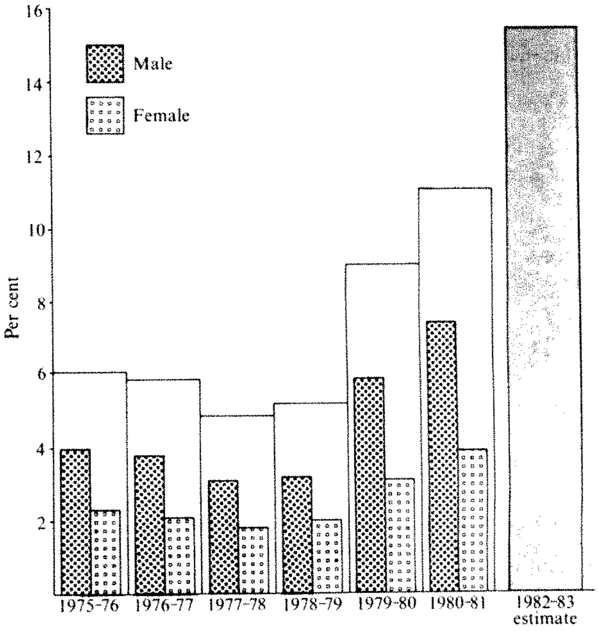
Unemployment continues to be highest amongst those reading arts subjects, and of the sciences biology clearly has the largest proportion unemployed. However, factors such as falling oil prices have had an effect on recruitment even in traditionally "safe" subject areas; some university careers offices have reported problems among civil and mechanical engineers, for example. The number of companies that recruit on the "milk-round" of universities has decreased this year (although the decrease is not large), and there are other indications that the number of advertised vacancies for graduates has fallen over the past two years. The sudden growth in the number of sponsorship schemes since 1979 (see accompanying figure) accounts for a part of this decrease, and it seems that the number of vacancies has probably reached a plateau. It is unlikely to increase significantly for at least 18 months, even if there were to be a major reflation of the economy next year. **Tim Beardsley**

Per cent home first graduates unemployed, shown according to discipline



A Architecture and other professional and vocational studies
B Agriculture, forestry and veterinary science
C Medicine, dentistry and health
D Education

Home first degree graduates believed unemployed at 31 December



All values are expressed as percentages of the total number of graduates of known destinations. About 10 per cent of graduates did not reply to survey questionnaires. The 1982 figure is an informed estimate.

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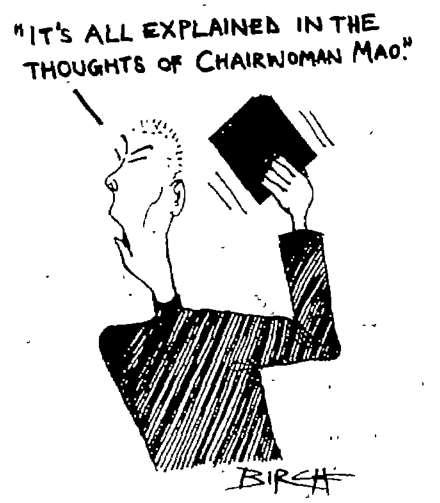
CORRESPONDENCE

Orang is man

Sir — I have long resisted the temptation to point out the erroneous practice of many Western editors and authors of abbreviating 'orang' for 'orang-utan'. Reference to any Malay or Indonesian dictionary will confirm that 'orang' means 'man' and 'orang-utan' means 'orang-utan'. Therefore, when Lowenstein, Molleson and Washburn reported that they have results to prove that 'both the Piltdown jaw and the canine tooth are those of an *orang-utan*' and you have their letter headlined "Piltdown jaw confirmed as orang" (*Nature* 23 September, p.294), I hope you are guilty only of misleading your readers with an inappropriate abbreviation and not of misunderstanding the key issue of the debate!

J. Fleuris

Quebec, Canada


"IT'S ALL EXPLAINED IN THE THOUGHTS OF CHAIRWOMAN MAO!"
BIRCH

Chinese puzzles

Sir — In *Nature* of 16 September (p.200) J.A. Irving wrote to point out that Chinese surnames precede given names. This is no longer a problem in English translation because given names are now hyphenated and the second one is not capitalized. For foreigners, however, the gender of nouns and pronouns remains confusing. For instance, "Mr" Qi, cited by Irving, is actually Mrs Qi.

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War prevention

Sir — Jeffrey Segall (*Nature* 4 November, p.10) attributes to me (*Nature* 7 October, p.482) approval of the Medical Association for the Prevention of War (MAWP) proposals for a "Comprehensive ban on all methods and means of warfare specifically designed to kill or injure by inducing human disease". He fails to see two separate strands in my comments on MAWP.

Firstly, I consider the existing international prohibitions and other restrictions on warfare as among the highest achievements of man. We should all hope for a wider and more effective extension where appropriate of their type of international agreement. However, without diminishing their significance, it should be recognized that their success lies in that they involve noncentral areas of military concern.

Secondly, we should not restrict our attention only to the salient and direct causes of suffering involved in war. The concern with the methods and means of warfare should not distort our perception to ignore the fact that wars are initiated in the pursuit of military and political goals. And that prevention and control of these goals are as significant, if not more so, as that of their means for stopping war and the suffering within them.

Unfortunately not all desirable things are compatible. The inhibition of the initiation of wars depends largely on deterrence which requires armaments. The removal of the world's dependence on deterrence is likely to be achieved not by disarmament but by either an ascendancy of one of the superpowers to be effectively the only world power, or the creation of internal political structures of inhibition within military superpowers. But the latter and most desirable change would require the unlikely development of political pluralism, autonomous judiciary and intellectually open societies in the at present totalitarian powers of the Soviet Union and China.

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Malaria resurgence

Sir — Chapin and Wasserstrom[1] stated that resurgence of malaria in India was the result of the introduction of high yielding varieties (HYV) of rice and cotton, and we disputed the basic tenets of this hypothesis[2]. The main thrust of their argument now, however, seems to be that resurgence was the result of the use of insecticides in agriculture[3]. We would like to add a few further comments.

(1) Widespread resistance to common pesticides in anophelines developed during early and mid-1960s. *Anopheles culicifacies* was found resistant to DDT in 271 units in 14 states and to BHC in 24 units in 4 states. Similarly *Anopheles stephensi* was found resistant to DDT in 34 units in 5 states and to BHC in 6 units in 3 states[4].

(2) *A. stephensi* type form, the vector of urban malaria in India, breeds in cisterns, overhead tanks, garden tubs, fountain basins, disused containers, receptacles used for storing water, and other water accumulations in cities and does not breed in agricultural fields. Resurgence of malaria was observed in cities of India from southern, central and north India[5].

(3) Although accurate quantities of DDT used in agriculture (1960–72) are not known, the available figures need not be extrapolated as they are about correct with minor variations.

(4) It is usual for genetic mutations for insecticide resistance to be less "fit", and reversion towards susceptibility generally occurs in the absence of insecticidal pressure. This was observed in *A. culicifacies* after withdrawal of spraying or change of insecticides in Maharashtra state in 12 units (28 villages)[4].

(5) Irrigation schemes contributed to the proliferation of the existing mosquito population which had become resistant in large parts of the country. It is incorrect to assume that the process of selection started *de novo* in the newly irrigated fields.

(6) In certain areas, a certain degree of contribution of the use of insecticide in agriculture in accelerating the pace of resistance in vectors of public health importance is understandable, and examples can be found to support this view. But it is difficult to believe that the massive quantities of insecticides used in public health and directed towards the malaria vectors were not the primary cause of widespread insecticide resistance.

The doubts raised by Chapin and Wasserstrom about the value of high-yielding crop varieties are a serious matter for a developing world striving hard to increase food production by introducing such varieties. The singling out of these crops as the main culprits for the resurgence of malaria without any authentic data is unjustified, and has been rightly condemned by Bruce-Chwatt as a "garbled account"[6].

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Muscling in

Sir — It is very misleading for muscle biophysicists to continue to refer to the anterior byssal retractor muscle (ABRM) of *Mytilus* as an example of unstriated (=smooth) muscle (*Nature* 299, 308; 1980). For almost 30 years, since the ABRM was first examined by electron microscopy, it has been realized that this muscle in fact enjoys a very regular, striated, ultrastructure. There is a highly orderly arrangement of thin and thick filaments, although they are arranged obliquely rather than transversely, in the relaxed muscle. The correct designation for this type of muscle is *obliquely-striated.*

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NEWS AND VIEWS

More speculation about oncogenes

Last week's reports of the surprisingly simple difference between a normal human gene and its equivalent in a human cancer cell is not merely important but provoking. What will happen next?

What follows is pure speculation. Those of an austere temperament should turn the page. Others, especially armchair molecular biologists, may welcome an opportunity to compare their private musings with the public hostages to fortune below. But in reality, nobody needs to be ashamed, for the discoveries described last week lead directly to a paradox.

The discoveries[1,2] are concerned with the difference between a normal human gene responsible for the production of a protein called p21 and the corresponding gene in cells derived from a bladder carcinoma; the difference consists of the replacement of one nucleotide by another precisely thirty-five units from the point in the functional gene at which the transcription of DNA into RNA begins. So the presence of the abnormal gene is a sign that cells derived from the bladder cancer have the properties of cancer cells, but not of course a proof that this simple mutation caused the malignancy in the first place. (This does not diminish the importance of the discovery, whose immediate interest is that it points directly to the protein p21 as one whose inknown function must play a crucial role in the biochemical functioning of both normal and malignant cells.) But it is also known that the mouse cells used as touchstones of carcinogenesis, peculiar though they may be, are also made cancerous ("transformed") when they are dosed with the normal gene for p21 provided that it is suitably equipped with the nucleotide sequences known to be necessary for viral replication in a vertebrate cell. The paradox is merely the question why the production of a small amount of an abnormal protein should have physiological consequences very similar to those of the production of an abnormally large amount of the normal protein.

Paradox

A paradox is a conflict between the conclusions of two apparently logical arguments. Paradoxes are usually resolved by the discovery that one argument or the other contains a hidden flaw, often of a semantic character. Can the Weinberg-Barbacid paradox be as easily resolved? That could be most simply accomplished if a little of the abnormal version of p21 and a lot of the normal protein were not different from each other but identical, at least in respect of the cancerous properties of the transformed cells. But how could that be? Both groups reporting last week point out that the effect of the genetic mutation they have found is that the simple amino acid glycine at position twelve in normal p21 is replaced by valine in the abnormal version, from which they infer that the altered protein molecules are less likely than normally occuring p21 to begin (from the end carrying a free amino-group) with a tidy coil of roughly helical structure.

This comparison connot however be exact. Even the tidiest helical protein structure is not helical forever. It must be supposed that from time to time the helical ends of normal p21 will be unwound, or that there will be some temperature-dependent proportion of normal p21 molecules that have the configuration now inferred for the abnormal variety. So is it possible that the similarity of the effects of small amounts of abnormal protein and large amounts of the normal variety, at least in respect of their cancerous properies, arises because large amounts of the normal protein provide small (but "sufficiently large") amounts of the abnormal configuration? That would be a semantic resolution of the paradox.

This speculation (it is not an argument) then raises the intriguing possibility that normal p21 in normal cells has two distinct functions, one involving the more common tidy helical structure of the first score or so of amino acids, the other requiring a structure similar to that inferred for the abnormal protein. So far, so good, but even speculation now abruptly stops. The normal function of normal p21 is entirely obscure, so that it is profitless to go guessing at the abnormal function. Fortunately, real molecular biologists will not be at a loss. Indeed, one of the obvious lines of enquiry now suggested is to use the techniques of genetic manipulation to right the changes on the first dozen or so amino acids of p21 to discover which parts of the structure are essential for the transforming activity of the abnormal gene. Telling how that part of the molecule functions will require more luck.

Evolution

This line of speculation leads to another. If the presence of an abnormal gene for p21 in a human bladder cell can have such profound effects, and if the frequency of radom mutations is as great as often supposed, by what means in the evolution of vertebrates has it been arranged that these lethal mutations are selectively suppressed? The question is more taxing than that of understanding the conservation of other genes that have survived long stretches of evolution more or less unchanged, for then it is at least possible to suppose that a cell inheriting an unwanted mutation will simply die unnoticed. But if taken at its face value, a mutation at position 35 of the p21 gene (or presumably at the preceeding one as well) will transform the cell which carries it into a form that grows successfully, and which proliferates. So it is natural to postulate some biochemical mechanism for removing these lethal mutations before than can do much damage.

This, of course, is not a novel speculation. Armchair oncologists were onto it while molecular biology was in its infancy. But two plausible conclusions follow. First, if the difference between a normal and a cancer cell is as delicate as now appears, the case for an active molecular mechanism for removing unwated mutations is strenthened (which is not to suggest that it is within sight of being understood). And second, given such a mechanism, the occurence of the abnormal gene in cells taken from a human bladder cancer is much more likely to be the repository of the mechanism of cancer than its first cause. That is just as well, for it would otherwise be hard to account for the long induction period associated with most forms of cancer, as everybody has pointed out.

The snag, with speculation in this or any other fields, is that it cannot solve puzzles but only replace one puzzle by another. The discoveries reported last week by the Weinberg and Barbacid groups, are themselves based on a new technology patiently developed over the past decade, and on a mass of painfully acquired and still poorly-assimilated information about the behaviour of retroviruses (which carry genetic information as RNA, but which can integrate as DNA among the genes of the cells that they infect). They are sensational because they are so unexpected and because they have been made at the bench, not in the armchair. That is also how the next surprises will arise in this now exciting chase. They will not be long delayed.

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Natural killers to rescue immune surveillance?

from Barry R. Bloom

Despite the myriad difficulties in finding definitive experimental verification, the idea of immunological surveillance holds a unique appeal for cellular immunologists. The existence of a surveillance mechanism for the continuous and systematic rejection of altered body cells was first inferred when it was found that specific cytotoxic T lymphocytes (CTLs)[1] could lyse allogeneic (non-self) cells and syngeneic (self) virus-infected or tumour cells. Soon apparent, however, was the disconcerting fact that athymic nude mice, which lack this mechanism, had no greater incidence of primary spontaneous, virally or chemically induced neoplasia than normal mice. It is in this context that the discovery of natural killer (NK) cells — present in both athymic and normal mice — renewed hopes that immunological surveillance might be a real phenomenon.

Natural killer cells represent an apparently heterogeneous population of cells with the capability of spontaneously lysing a variety of transformed cells, virus-infected cells and embryonal cells *in vitro*, without evident prior exposure to specific antigen *in vivo*. The revelation that NK activity is regulated by interferons, and also by interleukin (IL)-2, markedly heightened interest in these cells, if not their credibility.

Despite the extraordinary amount of information generated about NK cells in a very short period of time, recently reviewed at an excellent workshop* on natural killing, the most fundamental questions — the provenance and lineage of NK cells, their mode of recognition and their function *in vivo* — remain problematic.

The dogma implicit in much of cellular immunology, 'By their markers, shall they be known', is currently being confounded by NK cells. A large number of surface determinants have been identified on NK cells and have helped to establish their heterogeneity and define subsets, but most, if not all, of the markers are found on other cell types as well. In the mouse, NK cells, although lacking the Lyt 1 and Lyt 2 markers of helper and suppressor/cytotoxic T subsets, do share Thy 1, Qa4 and -5, Ly5 and asialo-GM$_1$ with T cells. Human NK cells express the OKT3, OKT6, OKT11 and to a lesser extent the OKT4 and OKT8 markers associated with immature and differentiated T cells. However, they also express the OKM1 marker found on monocytes and granulocytes. Murine NK cells express, in addition to Fc receptors for IgG, high-affinity IgE receptors, similar to mast cells and basophils. Worse yet, in short-term culture, the expression of markers can vary qualitatively from day to day[1]. Even the most useful and specific monoclonal antibodies (anti-NK1.1 in the mouse and HNK-1 [Leu/] in man) react with a greater proportion of cells than can be accounted for by NK activity. The recent observation (Stutman, Sloan-Kettering Institute) that one NK cell subset, termed NC cells, known not to be affected by interferon, is regulated by IL-3 which is also a mast cell growth factor, raises the possibility that specific growth factors may ultimately be helpful in resolving questions of NK cell lineage.

One of the controversies that has smouldered over the years is the relationship between NK cells and K cells, the latter being defined as cells possessing Fc receptors for IgG that have the ability to lyse antibody-coated target cells *in vitro*. These cells express almost all known NK markers yet clearly have a different mode of recognition and killing of target cells. Using the single-cell cytotoxicity assay, individual cells were shown to kill both NK-susceptible and antibody-coated targets, demonstrating that the same cell could carry out both activities[2]. Only in the germ-free piglet does that dichotomy remain (Kim, Sloan-Kettering Institute).

The selectivity or specificity of NK cells for certain target cells remains enigmatic. Many cloned NK lines have the ability to kill a wide variety of targets, including some not recognized by primary NK cells, yet some human clones have quite remarkable selectivity for only certain targets. It is unclear why some tumour or virus-infected cells are not lysed, and why some embryonal and normal cells are either killed or are capable of blocking killing of appropriate NK targets. Wigzell (Karolinska Institute) reported the intriguing finding of NK-like activity in two of six rat tumour-specific CTL lines. The cells had classical MHC (major histocompatibility complex) -restricted killing of the specific syngeneic chemically induced tumour target, but in addition could lyse xenogeneic NK targets (targets from a different species). An anti-idiotypic antibody was obtained which blocked the MHC-restricted tumour-specific killing, but did not affect the killing of the NK targets. This clearly indicated that recognition of the tumour-specific antigen and NK target structures are independent, and that receptors for NK targets are distinct from those for MHC-restricted specific antigens. It will be of interest to know whether exposure of these cells to the NK targets will clonally expand them for tumour-specific killing. Recently, human NK-like clones derived from mixed lymphocyte culture were found to have clonally distributed receptors distinct from MHC recognition[3]. These and most other experiments on NK cell recognition raise the question of whether there is a clonally distributed diversity of NK receptors for various target cells, or whether there is a class of surface structures common to some tumour cells, virus-infected cells and embryonal cells recognized by most NK cells.

If the mode of NK cell recognition remains mysterious, the mechanism by which they kill their targets is being solved, to the extent that they have become the paradigm for understanding cell-mediated cytotoxic mechanisms. Human and rat, but to a much lesser degree mouse, NK cells appear as large granular lymphocytes (LGLs), morphologically quite distinct from other lymphoid cells. A wide variety of studies ranging from microcinematography to the use of selective metabolic inhibitors indicate that lysis is mediated by vesicular secretion. Thus drugs that block vesicular secretion in other cell types, for example, mast cells, inhibit NK cell killing[4,5]; agents that degranulate, such as Sr^{2+}, both deplete the granules from LGLs and inhibit killing[6]. The stimulus–secretion model, initially suggested for NK cells by Roder, would hold that the critical requirements for killing are (1) binding to target cells, (2) movement of the uropod containing the granules into apposition to the target[7], (3) Ca^{2+}-dependent degranulation or release of an NK cytotoxic molecule, (4) binding of the cytotoxic factor to the target and (5) lysis of the target by the released factor. An NK cytotoxic factor has been found to be released from mouse NK cells and human LGLs on interaction with NK-susceptible targets[8,9], which has the ability to lyse NK-susceptible as well as some resistant targets. Target cells can be resistant to NK killing if they either fail to bind NK cells, or fail to bind or succumb to the cytotoxic factor. Because several laboratories failed to find production of oxygen radicals or inhibition of NK killing with radical scavengers, a proposed role for oxygen metabolites in mediating NK killing[10] has been met with some scepticism. If there are independent receptors for binding of NK cells and an NK cytotoxic factor, these mechanistic studies can explain some of the apparent selectivity for killing various target cells. From these studies, one hopes it will also be possible to define the nature of the NK cell defects found in several disease states.

To achieve credibility NK cells must be shown to perform some important functions *in vivo*. Many years ago, the late Gustavo Cudkovicz and collaborators[11] found a genetic association between NK activity and the acute rejection of allo-

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*'Workshop on Natural Killing' held at Quail Roost, North Carolina on 31 August–3 September 1982 was organized by H S Koren and R B Herbermann

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geneic bone marrow grafts. The antigen that was recognized in this system was found to be closely linked to the *H-2D* locus. Studies on this haematopoietic histocompatibility locus gave rise to the notion that NK cells may be important not only for acute rejection of bone marrow and other grafts, but also for maintaining regulation or surveillance of the bone marrow itself.

Genetically NK cell-deficient *beige* mice as well as mice selectively depleted of NK cell function by treatment with antibodies to NK1.1 antigen, asialo-GM1, or mouse interferon are found to have increased metastases of a variety of transplanted tumour cells, suggesting that NK cells have a role in restricting metastases. It has been very difficult to show, however, even in *beige* mice, that NK cells have a significant role in restricting spontaneous viral or chemical tumorigenesis. One difficulty in these studies is that almost all chemical and viral carcinogens profoundly depress NK cell function, often for very long periods of time. In a recent issue of *Nature* (4 November, p.31), Warner and Dennert provide compelling evidence that NK cells can mediate three major surveillance functions *in vivo* — rejection of bone marrow grafts, restriction of tumour metastases and inhibition of primary tumorigenesis. Dennert was the first to report continuous cloned cell lines with NK-like activity, and the ability of cloned NK lines to mediate these three surveillance functions *in vivo* suggests that the power of cloned cell lines will be no less great in providing a unique tool for studying mechanisms and functions of NK cells than it has been in the case of B and T cells. While questions remain whether the effects are mediated directly or indirectly by the cloned cell lines, and what the appropriate growth factors are that maintain the cells in an active state *in vivo*, these experiments provide direct evidence that NK cells can exert a profound regulatory role in acute graft rejection and in restriction of neoplasia and metastases. They provide an important model for exploring the role of NK cells in controlling viral, bacterial and parasitic infections *in vivo*. They will also be cited to justify the possibility of ultimately (in immunology, that means within two years) intervening therapeutically in human disease using purified LGLs or cloned NK cells. □

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Call *Alvin* for hot science

from Roger N. Anderson

RECENT discoveries of deep-ocean hydrothermal vents in the Mariana Trough back-arc basin have stirred new excitement within the geological and biological communities. In the past few years, hot springs billowing forth metal-laden waters at temperatures up to 350°C have been found all along the East Pacific Rise crest from 21°N to 12°N and 20°S. Fantastic and unique biological communities live near these hot springs, ingesting sulphur-eating bacteria. But to date, all these organisms and associated 'black-smokers' have been found in the eastern Pacific close to a mid-ocean ridge. Now a new hot-spring vent area has been found — not only is it in the western Pacific but it is also above a subduction zone, where the Pacific Plate is being forced under the Philippine Plate. Both the biological and geological settings are in distinct contrast to the eastern Pacific hot vents. Scientists are clamouring to document similarities and differences between the mid-ocean ridge hot vents and the back-arc basin vents.

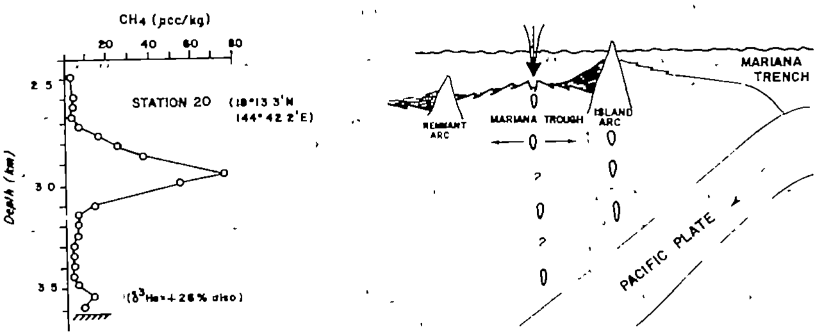
But first the evidence. As with the previous hot spring discoveries[3,4], oceanic heat flow measurements were the first to suggest hot-vent activity in the Mariana Trough[5]. Very hot patches of sea floor emitting more than two watts of geothermal power per metre (50 times normal) and bizarre temperature-depth recordings were strong hints of hot spring activity. A deeply towed acoustic imager with supporting temperature sensor next identified hot water[6] (admittedly only 0.1°C hotter than surrounding ambient seawater) associated with volcanic activity at the crest of the back-arc basin 'rift valley.' Metal deposits usually associated with massive generation of sulphide ore at sea-floor hot springs were also dredged. Now, Yoshio Horibe of the Ocean Research Institute, University of Tokyo, and Kyung-Ryul Kim and Harmon Craig of the Scripps Institution of Oceanography have uncovered conclusive evidence for the existence of hot vents in the Mariana Trough[7].

Hot vents carry rare gases from the magma below the sea floor to the ocean column. Thus if samples from the water column above suspected vent sites[3] are analysed, helium, hydrogen and methane anomalies can be found. This technique successfully searched out hot springs at 20°S on the East Pacific Rise.

Aboard the research vessel *Hakuho-maru* of the University of Tokyo, samples *m* from the back-arc basin spreading centre and from the waters above the two-watt power source were analysed for methane in February 1982. Methane is a clear product of hydrothermal vents in the eastern Pacific, but is consumed so quickly by bacteria floating in the ocean that a methane anomaly requires an active vent to spew hot hydrothermal waters into the ocean.

First, the scientists went to the two-watt area but no methane anomaly was found — the heat must be a remnant of a now-dead vent. Then at the back-arc basin spreading centre, the largest methane anomaly ever found in the oceans was recorded (see the figure). Later shore-based analysis verified this finding by showing a staggering 26 per cent increase in primordial ^{3}He. Hot vents must be active at the crest of the Mariana Trough back-arc basin.

Why the excitement? Vents are likely to exist all up and down the East Pacific Rise crest. Consider the biology. Will any living creatures be found? If so, will they be similar to those in the eastern Pacific: the large eastern Pacific clams are a form of mollusc indigenous to the northeastern coast of North America. Will the Mariana Trough have 'Japanese' clams? Will the spectacularly coloured tube-worms be



Schematic drawing of Mariana Trough back-arc spreading basin with methane anomaly measured in water column above rift valley (arrowed) by Horilu *et'al.*[7].

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present? Jack Corliss of Oregon State University has even postulated that life on Earth originated at 'black-smokers'!

Now to the geology: the subduction zone clearly supplies volatiles (for example, water to the volcanoes of the island arcs). Does the addition of this volatile source to the back-arc basin spreading centre change the chemical composition of the hot springs? And if so, does it change the metallogenesis occurring at spreading centres versus that at back-arc basins? These hot vents produce a significant quantity (about 20 per cent) of the mined metals in the world. Here is a chance to see the formation of metals in action today.

What next? Now it is time for the diving submarine *Alvin* to sample these Mariana Trough hot vents. Plans are being formulated for a diving season in winter–spring 1984–1985 in the western Pacific. Those of you who might have unique science to offer to the exploration of the Mariana Trough hot vents should contact the *Alvin* Review Committee, University National Ocean Laboratories (UNOLS) Office, Department of Oceanography, University of Washington, Seattle. □

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Gene conversion between repeated genes

from Thomas Petes and Gerald R. Fink

In the now 'classical' studies of gene conversion, the conversion of one gene to another occurs only among allelic genes present in single copies on homologous chromosomes. Recent work indicates, however, that among repeated genes, conversion can occur even when the genes are located on non-homologous chromosomes. In this article, we will discuss both examples of gene conversion and also the recent suggestion from Egel[1] and Baltimore[2] that gene conversion events occur not only in fungi but also in the cells of higher mammals. We consider that gene conversion in fungi and recombination events in mammalian cells might well be different.

Fungi are particularly suited to the study of gene conversion because all the progeny from a single fungal meiosis are sequestered in a structure called the ascus. For a heterozygous pair of alleles *A* (wild type) and *a* (mutant), the expected pattern of meiotic segregation is *2A* segregants and *2a* segregants. Gene conversion is detected by deviations of the type *3A:1a* or *1A:3a*. In tetrads with *1A:3a* segregation, Roman[3] showed that all three mutant alleles were identical. Thus gene conversion represents non-reciprocal precise transfer of information between two genes, either from *A* to *a* or from *a* to *A*. Extensive genetic analysis of this process has resulted in several other generalizations. For all loci, conversion can occur either from mutant to wild type or wild type to mutant. In *Saccharomyces cerevisiae*, conversion is often equally frequent in both directions, although significant deviations in one direction have been seen[4] Meitoic gene conversion in *S. cerevisiae* occurs but at a frequency of about one per cent per locus. Mitotic gene conversion also occurs but at a much lower frequency (10^{-4} - 10^{-6}). Deletions and insertions are converted at frequencies comparable with those found for point mutations[5]. For all organisms where genetic analysis has been possible conversion is non-randomly associated with reciprocal recombination[6].

Holliday[7] suggested that gene conversion is the result of heteroduplex formation between allelic genes, followed by mismatch repair. Mismatch repair involves the excision of one strand of the heteroduplex followed by resynthesis using the unexcised complementary strand as a template. This mechanism allows the faithful transfer of information from one gene to another. Holliday and others[8] have proposed that heteroduplex formation, which creates a potential site for gene conversion, is an obligatory intermediate in the process of reciprocal recombination.

Recombination events between single-copy genes are restricted to events between equivalent sites on homologous chromosomes, but repeated genes on nonhomologous chromosomes should also be capable of recombinational interactions. Using recombinant DNA procedures and the yeast transformation techniques, Scherer and Davis[9] constructed haploid *S. cerevisiae* strains with two copies of the *his3* gene (each copy containing a different small deletion); the genes were located on nonhomologous chromosomes. Since the wild-type *his3* gene was formed without concomitant formation of the double mutant, they concluded that His$^+$ recombinants were generated by gene conversion. Similar procedures were used to show that mitotic gene conversion occurs between repeated genes on the same chromosome[10]. Gene conversion has also been seen between naturally occurring repeated yeast genes. Ernst *et al.*[11] showed that some revertants of iso-1-cytochrome *c* mutations were the result of gene conversion between the partially homologous iso-1- and iso-2-cytochrome *c* genes. All these examples of gene conversion between repeated genes in *S. cerevisiae* are mitotic events but meiotic intrachromosomal gene conversion has also been demonstrated[11].

Meiotic gene conversion between repeated tRNA genes in the fission yeast *Schizosaccharomyces pombe* is reported by Munz and his colleagues in this issue of *Nature* (p.225). They show that an inactive UGA-reading serine tRNA suppressor gene (the suppressing activity lost because of a secondary mutation in the tRNA gene) can be re-activated by a recombination event. Assuming that all the secondary inactivating mutations used in the study do not have an extremely high rate of reversion, Munz *et al.* suggest that the inactivating mutations are removed by conversional interactions with other serine tRNA genes in the genome — one of these is on the same chromosome as the serine suppressor tRNA, the other is on a different chromosome. In support of these conclusions, they show by Southern blot analysis and DNA sequencing that serine tRNA genes can be altered in a single genetic event at multiple positions within the gene. Such alterations are much more easily explained as gene conversion events than as mutations. Remarkably, there is less than 200 base pairs of homology between the interacting tRNA genes which undergo this conversion event.

Gene conversion events between repeated genes, such as those described above, are likely to have two important genetic consequences. First, continued cycles of gene conversion within a family of repeated genes could lead to the maintenance of sequence homogeneity within that family[14]. Second, gene conversion can produce sequence diversity since conversion between repeats that differ at more than one position can form new combinations of sequences[1,2,9-12]. The net effect of conversion is likely to be a complicated function of the rate of conversion relative to mutation, the location of the interacting repeats, the length of the repeats and other factors.

Since gene conversion between repeated genes is likely to have important genetic consequences, it is obviously of interest to determine whether this type of conversion occurs in higher cells. Slightom *et al.*[15]

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found that the duplicated human γ^A and γ^G genes within a single chromosome were more similar than two allelic γ^A genes. They proposed that intrachromosomal gene conversion was the most economical explanation of the data. Similar observations have also been made recently for several other classes of repeated genes in higher organisms[1,2,16].

We believe that gene conversion may explain some recombination events between repeated genes in mammalian cells, but the process is yet to be demonstrated experimentally and may be different from that found in fungi. The observations in higher eukaryotes can be explained by unequal double cross-overs as well as by conversion. So far, no one has developed a system in higher organisms that allows examination of both participants in the recombination event before and after the postulated conversion. In considering gene conversion as a mechanism that maintains homogeneity among repeated genes[14], it is important to remember that conversion in fungal systems is often associated with reciprocal recombination[6]. It is not clear, therefore, how frequent cycles of gene conversion would be dissociated from the production of chromosomal deletions, inversions and translocations. This problem should be particularly acute in mammalian genomes where repeated sequences constitute a large fraction of the DNA. It is possible that in mammalian cells gene conversion is not obligatorily associated with reciprocal recombination. Even in fungi there is a class of mitotic gene conversions that are not associated with reciprocal recombination[10,17,18]. Over the next few years, it is likely that a combination of recombinant DNA techniques and DNA-mediated transformation will help to determine whether the recombination events observed in mammalian cells have all the properties of gene conversion events in fungi. □

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The origin of tektites — settled at last?

from Peter J. Smith

TEKTITES, those small glassy objects limited to a few well-defined regions of the Earth's surface, have haunted geologists for more than a century. Like country house ghosts, they have never been part of the investigative mainstream but have given rise to a variety of weird and wonderful explanations that attract the occasional attention of the wider community particularly when it has seemed that a new technique might throw some light on their nature and origin. Unlike country house ghosts, on the other hand, they are undoubtedly real, having been regarded as fine ornamental material since at least the time of Cro-Magnon man, about 31,000 years ago.

It was perhaps unfortunate that early scientific attention should have been directed towards Czechoslovakian tektites (moldavites) from a region of ancient European glassmaking. The result, during the nineteenth and early twentieth centuries, was a long time-wasting debate over whether tektites are real or artificial, productive only in the sense that the right conclusion was reached in the end. By then, however, it had already become clear that if tektites were natural they were not to be easily explained, for there was no obvious source, or 'mother lode'. The way was thus open to a wide range of possible explanations from the down-to-earth (literally and figuratively) to the bizarre.

Terrestrial volcanism was the obvious first choice; but it soon became evident that there were significant chemical differences between tektites and obsidian, the more common glassy material of undisputedly volcanic origin. Besides, candidate volcanoes close to the tektite zones, or 'strewnfields', were hard to find. To avoid some of these difficulties, Dunn (*Victoria, Geol. Surv. Bull.* **27**, 3; 1912) proposed an elaborate derivation of tektites from obsidian, involving lightning strikes in a very dusty atmosphere packed with hot gases, but it was not sufficient to overcome the objection that tektite compositions were far removed from those of terrestrial volcanics generally. During subsequent decades, tektites were to be attributed to, among other things, the heating of stony meteorites, the collision between a meteorite and a natural Earth satellite, the drying-out of siliceous gels acted upon by humic acid, and the fall of antimatter, although neither these nor many other hypotheses stood the test of time, if they ever stood at all.

What we are left with in 1982 are just two contending hypotheses, both of which involve extraterrestrial bodies. According to one, tektites are volcanic products ejected from the Moon; and according to the other, they are fragments ejected from the Earth itself by cometary or meteoritic impact. As work on returned lunar samples has proceeded, a lunar volcanic origin of tektites has come to seem less and less likely, although there are those, most notably O'Keefe (*Geochim. cosmochim. Acta* **44**, 2151; 1981), who would strongly dispute that view. The alternative explanation, by contrast, has risen in popularity, bolstered no doubt by a natural desire to avoid exotic solutions wherever possible. The hot money these days is on the idea that tektites result from the impact melting of terrestrial rocks, expecially sediments; and the odds in favour of that must surely have risen with the publication by Shaw and Wasserburg (*Earth planet. Sci. Lett.* **60**, 155; 1982) of a detailed study of the world's tektites using Sm–Nd and Rb–Sr systematics.

There are four tektite strewnfields, within each of which the tektites have the same K–Ar and fission track ages. Thus the North American, European, Ivory Coast and Australasian tektites are about 35, 14, 1.3 and 0.7 million years old respectively. These ages represent the times at which the tektites were last molten; they say little about the types of rock from which the tektites were formed, how they formed, or whence they came. Fortunately, however, it is now possible to determine rather more, both comparatively and absolutely, about the provenance and evolution of rocks from the behaviour of the Sm–Nd and Rb–Sr decay systems.

As for the comparative, one way of expressing isotopic ratios is to use the ε notation. Thus ε^{Nd} (0) is the measured deviation (in parts in 10^4) of the $^{143}Nd/^{144}Nd$ ratio from the present-day chondritic value of 0.511847; and ε^{Sr} (0) is the deviation (also in parts in 10^4) of the $^{87}Sr/^{86}Sr$ ratio from a model undifferentiated mantle reservoir value of 0.7045. The point about ε values is simply that they are different for different rock types. Shaw and Wasserburg have measured ε values for geographically and geochemically representative samples from all four tektite strewnfields and find that ε^{Nd} (0) is always negative and ε^{Sr} (0) is always positive and large. These values are characteristic of old terrestrial continental crust. They exclude continental material which has been derived from the mantle very recently, for that has ε values close to zero; and they exclude oceanic crust, because that has positive ε^{Nd} (0) values and negative ε^{Sr} (0) values. The isotopic data thus match the chemical data, for, as many

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workers have pointed out since at least 1917, the chemistry of tektites resembles nothing so much as continental sediments, especially sandstones. There are no known lunar rocks having major and trace element compositions similar to those of tektites, and there are no known meteorites with these compositions.

The second important discovery made by Shaw and Wasserburg is that within each tektite group ε^{Nd} (0) is uniform whereas ε^{Sr} (0) is highly variable. This reflects what earth scientists already suppose about the different types of information provided by the Sm–Nd and Rb–Sr systems. Assuming that concentrations are high enough to measure, any radioactive decay system will give an age for the rock concerned, but the term 'age' needs careful interpretation. The major fractionation of Sm and Nd appears to occur during the partial melting of mantle material to produce crust, and the Sm–Nd system is relatively undisturbed by such processes as weathering and sedimentation. Moreover, Sm and Nd are refractory and thus not readily fractionated by differential volatilization on impact melting. The Sm–Nd age of a tektite should therefore represent the time of formation of the crustal material from which the tektite was ultimately derived, and uniformity of ε^{Nd} (0) within a single tektite group inspires confidence that the Sm–Nd system is dating precisely that event. The Rb–Sr system, by contrast, can be severely disturbed by sedimentation, volatilization, weathering and diagenesis. It is only to be expected, therefore, that a tektite group will provide a range of ε^{Sr} (0) values and Rb–Sr ages reflecting the varied history of the tektite source rocks and descending to the last major parent–daughter fractionation event. In short, if nature is behaving itself, all tektites within a group should have a common Sm–Nd age, a range of younger Rb–Sr ages and, of course, a common and even younger K–Ar age representing the time at which the tektite themselves formed.

And thus it turns out. The Sm–Nd ages obtained by Shaw and Wasserburg for the North American, European, Ivory Coast and Australasian tektites are, respectively, about 650, 900, 1,900 and 1,150 million years. In other words, all the ages are consistent with the tektites' having been formed from Precambrian crust. The Rb–Sr data then indicate what happened between the formation of that crust and its conversion to tektite. The European tektites, for example, were derived from sediments laid down only about 20 million years ago. Interestingly, sediments in the nearby Ries Crater have ε values and isotopic compositions similar to those of the tektites themselves. Moreover, the age of the oldest basement in the crater area agrees well with the Sm–Nd age of the tektites. The new data thus support an earlier suggestion that the Ries Crater was formed by the impacting body that also gave rise to the tektites.

WHILE watching the grand display of aurora on Friday night from our roof, at about 6h. 7m., my wife and I saw a strange gleam of light rising above a bank of cloud on the eastern horizon, nearly vertically below the Pleiades, like the gleam of another moon rising in a haze. It grew out slowly, as we watched it, into a strong beam of white light slanting towards the south, and we stood in wonderment as it lengthend out making straight towards the moon. Presently its tail was dis-engaged from the cloud, and it stole through the sky like a long luminous nebulous "cigar ship" exactly across the moon, and away down into the west, sinking as slowly as it had risen. You will probably receive many accounts of this strange apparition. It will be interesting to know the position relative to the moon in which it was seen by different observers. Was it clear of the earth's atmosphere or not?

HUBERT AIRY
Woodbridge, November 19, 1882.

The Ivory Coast strewnfield has also been associated with an impact structure, namely, the Bosumtwi Crater. The rocks around this crater have ages comparable with the Sm–Nd age of the tektites. The Rb–Sr systematics indicate that the tektites were formed from Precambrian sediments about 950 million years old, although the sediments have not been identified yet. The North American tektites, by contrast, have no known source crater. Moreover, the fact that the material that went to form the tektites was derived from the mantle as recently as the late Precambrian rules out most of the North American Precambrian shield, and the sediments derived therefrom, as a source region. Similar age criteria refute the recent suggestion from Dietz (*Meteoritics* **12,** 145; 1977) that the Popigai astrobleme of Siberia is the source of the North American strewnfield. The only known continental material of the required age is the late Precambrian crust constituting the east-southeast margin of the Appalachian orogenic belt, although no suitable crater has been identified within it.

Little more can be said about the origin of the North American tektites, except that the immediate source material was sedimentary, for the Rb–Sr proved unsuitable for the determination of an age of sedimentation. Not the least interesting conclusion to be drawn from the work of Shaw and Wasserburg, however, is that an oceanic impact for the origin of tektites is not ruled out by the restriction that tektite source material cannot be oceanic crust. Although the effects of the impact at the Ries Crater extend to depths of about 2 km, the tektites supposedly produced by the impact were formed from sediments which appear to have been only a few tens of metres thick at the time. The implication of this is that an oceanic impact could well generate tektites simply by sampling thin continentally derived sediments lying on top of the basaltic oceanic crust. In other words, an impact origin of tektitites is not precluded by failure to discover a suitable impact structure on land.

In the case of the Australasian strewnfield there are several candidate craters on land — the Zhamanshin and Elgygytgyn Craters of the USSR and an unnamed crater-like structure in Cambodia. Nothing much is know of the Cambodian crater and the Elgygytgyn Crater can be ruled out on age grounds. The most touted possibility is the Zhamanshin Crater, but the case for it is weak, if not nonexistent. For example, this crater is associated with tektite-like objects known as irghizites, which have chemical compositions similar to, but Nd isotopic compositions quite different from, those of the tektites of the Australasian strewnfield. It is possible in principle that both the irghzites and the Australasian tektites were produced at the same crater but that the irghizites managed to take up a proportion of the impacting meteoritic material, although Shaw and Waserburg show that it is impossible in practice to obtain the correct ε values for the irghizites simply by adding chondritic material to the tektitic. It seems much more likely that the impact body that supposedly gave rise to the Australasian tektites fell in the ocean; but wherever it struck, it generated tektites from continentally derived sediment about 250 million years old.

What is impressive about the story pieced together by Shaw and Wasserburg is its overall coherence and internal consistency. So can we take it that the tektite issue is now settled? Or will the lunar volcanologists fight back, as they have so often done before, with a quite different interpretation of the same data? We shall see. In the meantime, it could well be objected that, coherent or not, the cometary impact hypothesis is no less exotic a mechanism for the production of tektites than is lunar volcanism. But somehow it does seem less so these days. Recent calculations on the frequency with which comets large and small might be expected to reach the Earth's surface, and the remarkably cool way in which the earth science community has been willing to consider seriously the suggestion by Alvarez *et al.* (*Science* **208,** 1095; 1980) that cometary impact may have been responsible for the Cretaceous–Tertiary extinctions, suggest that the rigid anti-catastrophism characteristic of geological thinking since the time of Hutton and Lyell is now dead.

Yet at least one puzzle remains, and remains unremarked. If cometary impacts have been responsible for biological extinctions throughout the Phanerozoic, why have they apparently only generated tektites during the past 35 million years? Curious. □

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Learning, forgetting and the cell biology of memory

from Miranda Robertson

PERHAPS memory has proved such an intractable problem for so long because, like embryogenesis — the other central metazoan mystery — it is a property of interacting cell systems, depending on cellular signals whose mechanisms seem likely to be the last to yield to molecular analysis. Charles Stevens (Yale University), closing a recent meeting* primarily concerned with the biology of memory (but see box overleaf), argued that memory and development are not merely similar problems but one and the same. From this point of view, the traditional distinction between 'hard-wired' and plastic neuronal systems is reduced to a matter of timing: the final differentiation of some of the neurones in a plastic system is delayed until long after maturity.

Stevens's near-impossible brief was an overall synthesis of a conference that began with the properties of learning and forgetting in damaged and undamaged vertebrates and descended fairly precipitously to the properties of ionic channels in lower forms of life. Paul Rozin (University of Pennsylvania), discussing the adaptive properties of learning at the upper end of this spectrum, gave some arresting examples of plastic systems with a hard-wired basis. The most obvious of these is the imprinting of precocial birds, which follow and later make sexual advances to anything that looks like the first moving object they see (usually their mother); the responses in this case are hard-wired, only the stimulus being determined by experience. More subtle constraints may operate in mammalian learning: for example, a rat taught to associate a light and a specific taste with malaise will learn to avoid the taste, not the light.

One hope of those engaged in research on the neuropsychology of memory is to identify specific brain structures essential for its consolidation. Given that memory can be regarded as a property of any neural circuit whose activity is modifiable by experience, this may seem an optimistic aim. It is therefore perhaps surprising that damage to the medial temporal lobes of the human brain should have proved to be specifically associated with failure of memory for events occurring after the injury, in the absence of sensory or cognitive deficits. In the light of recent analyses, however, the memory deficit begins to look less general than it might at first appear. Brenda Milner (McGill University), for example, summarized recent evidence for lateralization: lesions of the left medial temporal lobe generally lead to failure of verbal memory, lesions of the right to deficits in memory for spatial position.

A more profound specialization still has been suggested by the research of Neal Cohen in the laboratory of Larry Squire (University of California, San Diego). He has demonstrated that medial temporal lesions affect only what he calls declarative learning — or 'learning that'; damaged patients are still perfectly capable of procedural learning — or 'learning how'. Thus for example, a patient with almost complete failure of verbal memory can be taught to read mirror-reversed writing and retain the skill on testing three months later, though he will not remember having been taught it[1]. This is a particularly telling example because reading reversed print is a verbal skill. Squire believes that procedural learning may be different in kind from declarative learning: it takes longer to acquire and is much less easily forgotten, as well as being resistant to brain damage affecting other kinds of memory. Whether this really reflects a difference in kind, or is a function of the number of neural pathways involved, is impossible to say.

Without doubt the most striking and provocative data to be reported at the conference were those of Fernando Nottebohm (Rockefeller University) on song learning in canaries, which is another clear case of partial plasticity. The song of male canaries has an invariant species-specific basis that is elaborated by learning, and the songs of individual birds will change considerably from one year to the next. The ability of the birds to learn new songs (though not their ability to sing) depends on a specialized neural centre known as the hyperstriatum ventrale, pars caudale (HVc). The size of the HVc, and that of a lower song control centre, the nucleus robustus archistrialis (RA), is correlated with the size of the birds' song repertoire — being very small in females, which are songless, and increasing in males during the spring, when testosterone levels are high and the season's song repertoire is growing. In the RA these size differences can be accounted for in part by differences in the size of the dendritic trees of the neurones[2] and it seems likely that similar changes occur in the HVc.

But recent investigations have suggested the more startling possibility that in the HVc the size fluctuations may be partly due to the continual recruitment and loss of neurones in the nucleus. The evidence for such turnover comes from experiments on female canaries. Under the influence of testosterone, the female HVc doubles in size[3]. In the light of this dramatic increase, Nottebohm, with his colleague Steven Goldman, went on to investigate whether testosterone might be inducing cell proliferation and found that new HVc neurones were appearing all the time even in the absence of the hormone. They are accordingly now monitoring the HVcs of both male and female canaries at monthly intervals to see if they can detect changes in the rate of cell division; and Nottebohm suggests that the addition and loss of neurones in the HVc reflects a continuous 'updating of the software' of song production — cell turnover in females reflecting, perhaps, the complementary need to recognize each new season's repertoire of serenades.

The implication of such drastic changes is that learning entails an irreversible change in the HVc neurones so that new learning requires new cells containing uncommitted DNA. If this is the case, there is certainly a radical difference between avian and human song learning; but so little in known of the cellular basis of memory that the possibility cannot be dismissed.

It is generally assumed that learning entails short-term changes in the activity of groups of neurones, leading to longer-term or effectively permanent changes in synaptic resistance. Evidence from the effects of trauma (for example concussion or electroconvulsive shock) suggests that while the short-term changes may have a time course of minutes to hours, longer-term consolidation may continue for up to two years.

Both Eric Kandel (Columbia University) and William Quinn (Princeton University) addressed themselves to the possible molecular basis of these changes in animals with simple nervous systems. Kandel has focused on the modulation of the gill-withdrawal reflex in the sea-snail *Aplysia*. This can be elicited by touching the animal's siphon, and enhanced by simultaneous stimulation of its tail. Kandel has shown that such sensitization, which can be regarded as a simple form of learning, depends on presynaptic modulation by serotonin of the activity of the sensory neurones mediating the reflex. Serotonin binds to receptors on the presynaptic nerve terminal, activating adenylate cyclase and leading to the cyclic AMP-dependent phosphorylation of a potassium channel in the presynaptic membrane. The resultant closure of the potassium channel prolongs the action potential, thus increasing the influx of calcium and the release of transmitter onto the postsynaptic cell[4,5]. The same mechanism has more recently been shown to underlie a simple form of classical conditioning in which a weak stimulus to the tail precedes the stimulus to the siphon.

Quinn has sought, by the analysis of

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learning mutants, to establish a similar kind of mechanism in *Drosophila.* One mutant, *dunce,* has a defective phosphodiesterase and thus presumably abnormal cyclic AMP metabolism[6]; a second, *rutabaga,* isolated by Patricia Szieber in his laboratory, has been shown by Margaret Livingstone to be deficient in adenylate cyclase activity. Quinn is now hoping to identify the neurotransmitter whose receptor is (presumably) coupled to the crucial adenylate cyclase, so as to explore further the possible parallel with *Aplysia.* Preliminary evidence implicates either dopamine or serotonin, which is particularly interesting in view of recent evidence that the distribution of dopamine is correlated in rat brain with that of a membrane protein whose phosphorylation can be shown *in vitro* to depend on the dopamine-activated synthesis of cyclic AMP[7].

Thus catecholamine-activated protein phosphorylation may be part of a general mechanism of memory. But how far can the phosphorylation of ionic channels explain the phenomenological properties of memory? Stevens, in his summary discussion, pointed out that it cannot on its own account for any memory lasting more than two or three weeks, which is the maximum lifetime of a membrane protein. If learning depends on the phosphorylation of membrane proteins, then neurones must be re-programmed to keep phosphorylating them. It was this kind of consideration that led him to suggest that memory can be seen as the last step in neural differentiation: a final change in gene expression that is reserved until after birth and is contingent on experience. Just as during development, a morphogenetic signal changes the state of differentiation of a cell and thus its responses to subsequent signals, so the early changes in neural activity that underlie short-term memory may render the neurone susceptible to later signals that induce a change in gene expression and long-term storage. There is in fact evidence that in some systems, neural morphogenesis is profoundly influenced by neural activity: learning may reflect the same (unknown) mechanism. Indeed changes in dendritic or synaptic morphology may be the basis for long-term memory.

An understanding of memory at this level will not, in Stevens's view, provide a complete understanding of the encoding of memories — any more than understanding the behaviour of transistors would help with the understanding of a computer program. But it could establish, for example, whether the process is reversible or not. It is possible that memories are for ever, and forgetting is just loss of access. Certainly this is to be expected from the developmental parallel, since differentiation is generally extremely stable, if not completely irreversible. There is, however, one notable case in which terminal differentiation entails the editing of the DNA and irrevocably limits the potential of the cell for further change. This is the case of the antibody-producing lymphocyte, in which recombination of the DNA is part of the basis for antibody diversity. Stevens, echoing Nottebohm's speculations, suggested that some similarly irreversible step might be made by the song cells of the canary HVc — so that the only way to change the repertoire would be by the loss of the old neurones and the addition of new ones.

It is however more likely (as he went on to emphasize) that versatility in memory depends not on the generation of diverse proteins by DNA recombination, but on the versatile deployment of a limited number of related families of receptor and channel proteins whose expression in different combinations confers on nerve cells the specialized properties of which so far we have such a limited understanding. Now that, with the cloning of the genes encoding the acetylcholine receptor[8], the first members of these protein families have yielded to recombinant DNA technology, dramatic advances in understanding their relationships and expression may not be far away. □

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Seeing in colour

THE showpiece of the First McDonnell Conference on Higher Brain Function was the spectacular re-entry into research on colour vision of David Hubel (Harvard University), who, in a kingfisher shirt and scarlet tie, and with the assistance of Margaret Livingstone manipulating coloured lights, gave a personal demonstration of the importance of context in hue perception. The demonstration is simple (and in principle well known): bathe the outrageous shirt and tie in red light and the outrage vanishes: the shirt becomes black while the tie assumes the same pallid appearance as the speaker's face; similarly in blue light the vividness of the shirt is drained. This illustrates that human colour vision is not a simple function of the wavelength of light reaching the retina — as any reader who has a copy of *Nature* for 3 April 1980 (and has not lost the colour filters supplied with it) can check for himself.

Among the best explanations so far offered for the relative indifference of the visual system to the absolute wavelength of the stimulus is Land's retinex theory (discussed in the same issue of *Nature*[1]), according to which the sensation of colour is the outcome of interactions between neurones that respond selectively to lights of different wavelengths. Specifically, the theory predicts the existence of cells whose receptive fields are so organized that they respond maximally when the centre of the field is illuminated by light of a wavelength complementary to that illuminating the surround. A highly effective stimulus would thus be a red dot in a blue surround. Neurones responding to such stimuli are known as double-opponent colour cells and have been detected in very small numbers in the lateral geniculate nucleus of the cat[2], which has relatively poor colour vision, and in the visual cortex of the monkey[3], whose colour vision is comparable with man's. This anatomical difference between cat and monkey reflects the general rule that sophisticated sensory computations tend to be postponed to increasingly central sites with ascent up the phylogenetic tree.

Hubel and Livingstone have now illuminated a further species difference by locating the cortical double-opponent cells precisely in the patches, or 'blobs', of exceptionally high metabolic activity recently revealed by cytochrome oxidase staining in layers II and III[4,5]. These patches have two curious features (discussed in *Nature* by Swindale[6]). First, they contain cells that, unlike most visual cortical neurones, are not selective for stimulus orientation[7]; and second, they are absent from the visual cortex of the cat.

The absence of orientation selectivity could be explained if the patches represented areas of convergence of orientation columns[6] — and thus containing neurones responding to all orientations. But then why none in the cat? A possible answer to this question now suggests itself: the patches may, instead of representing converging orientation columns, be colour columns; and their absence in cats probably reflects the relative crudeness of feline colour vision.

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ARTICLES

Glucocorticoid regulation of protein processing and compartmentalization

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Glucocorticoids appear to regulate the post-translational processing and compartmentalization of certain proteins in cultured rat hepatoma (HTC) cells. We have found that maturation of murine mammary tumour virus (MTV) proteins in infected HTC cells is hormone-dependent. Thus, MTV gene products may represent versatile and sensitive probes for examining cellular regulation of protein maturation.

THE location at which a given protein resides or acts is an integral and important aspect of gene expression. In general, proteins are 'targeted' or transported either during or after synthesis to particular intra- or extracellular compartments; the final gene product may be soluble in the cytoplasm, sequestered within specific intracellular organelles, inserted into particular cellular membranes, or secreted out of the cell. In addition, and perhaps related to this phenomenon of compartmentalization, many proteins are subject to one or more processing or chemical modification reactions including site-specific cleavage, phosphorylation and attachment of carbohydrates or lipids. Thus, the final location and biological activity of such mature proteins could in principle be regulated by modulating the appropriate post-translational events.

What signalling molecules might be involved in regulating gene expression at this level? In animal cells glucocorticoids and other steroid hormones act via specific intracellular receptors to elicit a broad range of responses in various target cells[1–3]; it has been established in some cases that the hormone–receptor complex directly stimulates transcription of the regulated loci. For example, when DNA encoding MTV is introduced into the genome of HTC cells, dexamethasone, a synthetic glucocorticoid, rapidly and selectively increases the rate of MTV RNA transcription[4,5]. Dexamethasone also regulates the expression of certain intracellular, cell surface and secreted proteins encoded by the HTC genome[6–13]; in these cases, the molecular processes under hormonal control have not been fully defined. Glucocorticoids could conceivably affect expression of some genes by stimulating or inhibiting transcription, others at a post-transcriptional event, and still others at multiple stages. To test this notion in detail requires the ability to detect specifically the DNA, RNA and proteins corresponding to a regulated gene and its products; unfortunately, such a combination of molecular hybridization and immunological reagents has not yet been reported for a glucocorticoid-controlled HTC cell gene.

In mammary tumour cells, MTV encodes two major polyproteins, each of which is specifically modified, cleaved and compartmentalized in an array of reactions that appear similar to those that effect maturation of various classes of cellular proteins[14–17]; specific antisera and cloned nucleic acid probes are available to monitor the precursor and mature MTV proteins, as well as the viral transcripts and genes. Therefore, MTV gene products in infected cells might be useful as general probes for putative glucocorticoid-regulated cellular functions that act post-translationally. Thus, we have used biochemical and genetic approaches to examine this possibility in an MTV-infected HTC cell derivative.

Effects of dexamethasone on MTV protein expression

Viral proteins were examined in M1.54, a cloned line of infected HTC cells containing 10 stably integrated MTV proviruses. As MTV RNA is produced at a basal rate in this line, and is strongly induced by dexamethasone[18,19], viral protein synthesis and maturation can be examined before and after hormone treatment. In addition, infectious MTV particles are not produced in this heterologous system[20], thereby precluding reintroduction of viral proteins into the cells via secondary infection.

In initial experiments, the full complement of MTV proteins produced by these infected rat cells was examined. M1.54 cells were labelled with ^{35}S-methionine for 12 h in the presence or absence of dexamethasone; hormone-treated cultures were exposed to dexamethasone for 24 h before labelling. Cellular and secreted proteins were solubilized in detergent-containing buffer and immunoprecipitated with an anti-MTV antiserum that detects all MTV gene products; to normalize for hormonal induction of viral transcripts, approximately equivalent amounts of immunoprecipitated radioactivity were then analysed on SDS-polyacrylamide gels. In hormone-treated M1.54 cells (Fig. 1*a*), 13 viral proteins were detected, ranging from molecular weight (M_r) 200,000 to 24,000, and comprising about 2% of the total labelled proteins. As shown below, the 74,000-M_r species actually represents two distinct viral gene products. In contrast, in the control cultures (Fig. 1*c*), seven of the MTV proteins were barely detectable or absent altogether relative to the remaining species, which were indistinguishable from those produced in dexamethasone-containing medium; the hormone-induced proteins are marked in Fig. 1 with arrows. Control experiments with preimmune sera (Fig. 1*b*, *d*) confirmed that all the proteins detected are MTV gene products.

Figure 1 shows that the 12-h labelling period enables even minor viral protein species to be detected; however, the possibility remains that the failure to observe certain of the proteins in the absence of dexamethasone is due to their relative instability, rather than their failure to accumulate. Therefore, cells were pulse-labelled with ^{35}S-methionine for 20 min then incubated for an additional 60- or 120-min 'chase' period in the presence of excess unlabelled methionine. The results (Fig. 2*a–f*) show that in the absence of dexamethasone, only the 74,000-M_r species is detected in either the pulse-labelled or pulse-chased material; in contrast, the 74,000-M_r protein pulse-labelled in dexamethasone-treated cultures is subsequently chased into the major viral products observed after long-term labelling (compare Figs 1*a* and 2*g–l*). While this experiment does not exclude extremely rapid degradation of the proteins in the control cultures, the simplest view is that they are produced only in the presence of hormone. Taken together, these results are consistent with the idea that glucocorticoids in some way regulate post-translational MTV protein maturation in M1.54 cells.

MTV transcripts in M1.54 cells

An alternative explanation for the hormonal effect on MTV protein expression is that dexamethasone-induced MTV transcripts differ qualitatively from those produced in the absence of hormone. Altered patterns of initiation and splicing or polyadenylation have been observed in other systems in response

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to changing physiological conditions or differentiation states[21,22]. By this view, hormonal regulation of transcription would account for qualitative and quantitative changes in MTV protein production.

Thus, MTV RNA was characterized biochemically and functionally. Total RNA from M1.54 cells cultured in the presence or absence of dexamethasone was translated *in vitro* in a rabbit reticulocyte lysate. Immunoprecipitation with anti-MTV antiserum and subsequent electrophoretic analysis revealed no effect of dexamethasone on the three MTV-specific polypeptides synthesized (Fig. 3*a–h*). In addition, cellular RNA from induced and uninduced cells was fractionated by agarose gel electrophoresis, transferred to nitrocellulose and hybridized with cloned MTV ^{32}P-DNA; the amount of MTV RNA in each gel sample was normalized. As shown in Fig. 3*i*, *j*, dexamethasone has no detectable effect on the sizes or relative stoichiometry of the two MTV RNA species produced in M1.54 cells, whereas no virus-specific sequences are detected in uninfected HTC cells (Fig. 3*k*, *l*). Further experiments have established that the hormone does not affect the site of initiation of viral RNA transcription in infected cells, nor does it alter either the sites or efficiencies of MTV RNA splicing and polyadenylation[23]. Taken together, these results show that the MTV RNA molecules produced in the presence and absence of dexamethasone in M1.54 cells are qualitatively and functionally identical, and that any effect of glucocorticoids on viral protein maturation probably occurs co- or post-translationally. Independent support for this view is provided by experiments demonstrating that an MTV precursor protein pulse-labelled with ^{35}S-methionine in untreated M1.54 cells is subsequently matured when dexamethasone is added to the medium during the chase period (G.L.F., unpublished result).

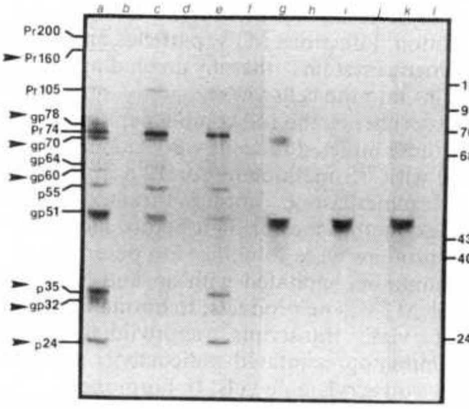

Fig. 1 MTV proteins produced by M1.54, and by a complement-resistant derivative, CR4. M1.54 and CR4 cells (5×10^6) were labelled with 100 µCi ^{35}S-methionine for 12 h in 4 ml methionine-deficient Dulbecco's modified Eagle's medium supplemented with 1% dialysed fetal calf serum; hormone-treated cultures were incubated with 1 µM dexamethasone 24 h before as well as during the radiolabelling period. The cells were collected by centrifugation (600*g*, 3 min), and the supernatant was re-centrifuged (10,000*g*, 10 min) to remove cell debris. The cell pellets were homogenized in 1 ml of 1% Triton X-100, 0.5% sodium deoxycholate, 5 mM EDTA, 250 mM NaCl, 25 mM Tris-HCl, *p*H 7.5 (solubilization buffer) and the solubilized proteins collected as the supernatant after centrifugation at 20,000*g* for 20 min at 4 °C. The medium was adjusted to 1% Triton X-100, 0.5% sodium deoxycholate, 5 mM EDTA just before immunoprecipitation. Viral proteins were immunoprecipitated by a modification of the staphylococcal protein A-antibody adsorption procedure[41]; 10-fold more extract from uninduced cells was immunoadsorbed, thereby approximately equalizing the amounts of MTV proteins analysed per sample. The detergent-solubilized fractions (300–500 µl) were preadsorbed with 10 µl of 10% fixed *Staphylococcus aureus* (Staph A) for 15 min at room temperature. After removal of Staph A by centrifugation, 1 µl of either anti-MTV rabbit serum (lanes *a*, *c*, *e*, *g*, *i*, *k*) or preimmune serum (lanes *b*, *d*, *f*, *h*, *j*, *l*) was added; antisera were given by R. D. Cardiff and co-workers[42]. The mixtures were incubated at room temperature for 15 min before addition of 10 µl of 10% fixed Staph A that had been preadsorbed with unlabelled cell extracts. After incubation for 5 min, the Staph A was centrifuged through 1 M sucrose in solubilization buffer, washed once in solubilization buffer and then once in 5 mM EDTA, 10 mM Tris-HCl, *p*H 7.5 (buffer B). Nonspecific binding of cellular radiolabelled proteins was significantly reduced by a second immunoprecipitation of the viral antigens. The immunoadsorbed antigens were eluted from the Staph A-associated IgG by incubating the Staph A pellets with 30 µl of 2% SDS at 37 °C for 20 min. After removing the Staph A by centrifugation, the eluted viral antigens were added to 400 µl of solubilization buffer containing 0.8 µl of antiserum and incubated at room temperature for 15 min. Fixed Staph A (10 µl of a 10% suspension) was added, incubated for 5 min and collected by centrifugation. The pellets were washed once in solubilization buffer and once in buffer B. Finally, the immunoadsorbed MTV proteins were eluted from the Staph A in SDS sample buffer, fractionated by SDS-polyacrylamide gel electrophoresis[43], and visualized by fluorography[44]. The figure shows cellular MTV proteins from hormone-treated M1.54 (*a*, *b*), uninduced M1.54 (*c*, *d*) and hormone-treated CR4 (*e*, *f*). Arrows denote the viral gene products produced in a hormone-dependent manner in M1.54. Also shown are secreted MTV proteins from hormone-treated M1.54 (*g*, *h*), uninduced M1.54 (*h*, *i*) and hormone-treated CR4 (*k*, *l*). The molecular weight markers are: β-galactosidase (M_r 120,000; 120K), phosphorylase *a* (94K), conalbumin (76K), bovine serum albumin (BSA; 68K), ovalbumin (43K), aldolase (40K) and chymotrypsinogen (24K).

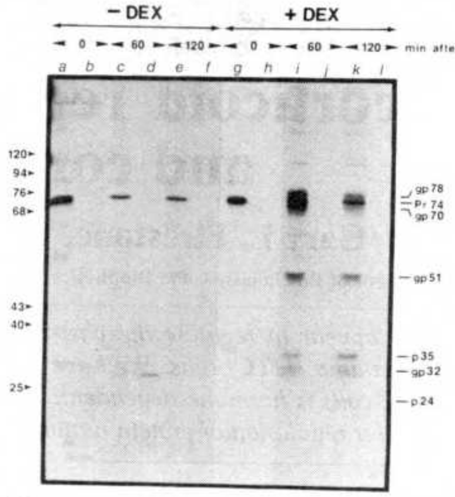

Fig. 2 ^{35}S-methionine MTV proteins produced by M1.54 during pulse and subsequent chase. M1.54 cells (10^7) were labelled at 37 °C for 20 min in medium containing 100 µCi ml^{-1} ^{35}S-methionine; cultures were either collected immediately or incubated for an additional 60 or 120 min in chase medium containing 2 mM unlabelled methionine. Hormone treatment, preparation of extracts, immunoprecipitation, gel electrophoresis and molecular weight standards were as described in Fig. 1 legend. Immunoprecipitation was with anti-MTV rabbit serum (lanes *a*, *c*, *e*, *g*, *i*, *k*) or preimmune serum (lanes *b*, *d*, *f*, *h*, *j*, *l*). *a–f*, Cellular MTV protein from uninduced M1.54 cells that were: *a*, *b*, pulse-labelled for 20 min; *c*, *d*, pulse-labelled and chased for 60 min; and *e*, *f*, pulse-labelled and chased for 120 min. *g–l*, MTV proteins from dexamethasone-treated M1.54 cultures that were: *g*, *h*, pulse-labelled for 20 min; *i*, *j*, pulse-labelled and chased for 60 min; and *k*, *l*, pulse-labelled and chased for 120 min.

Regulation of MTV phosphoprotein maturation in M1.54 cells

Two MTV transcripts similar to those described here are detected in virion-producing mouse mammary tumour cell lines; one specifies core proteins encoded by the *gag* gene, while the other specifies envelope proteins encoded by the *env* gene[24]. Each precursor acquires characteristic modifications; the *gag* polyproteins are phosphoproteins whereas the *env* polyproteins are glycoproteins. In both cases, the polyproteins are subsequently processed into several mature species. Thus, monospecific antibodies directed against either the MTV envelope glycoproteins or the core phosphoproteins were used to identify further the MTV proteins produced in M1.54 cells. This showed that the mature *gag*-encoded species are p55, p35 and p24, and that the mature *env*-encoded species are gp78, gp70, gp64, gp60, gp51 and gp32 (see Fig. 1 and below). In addition, the major precursors encoded by both the *gag* and *env* genes are M_r 74,000, and are designated as Pr74 in Fig. 1.

Immunoprecipitation with the monospecific antibodies also revealed that all the viral phosphoproteins in M1.54 are *gag*-related (data not shown); therefore, ^{32}P-phosphate was used to label selectively the *gag* proteins. Figure 4 shows that

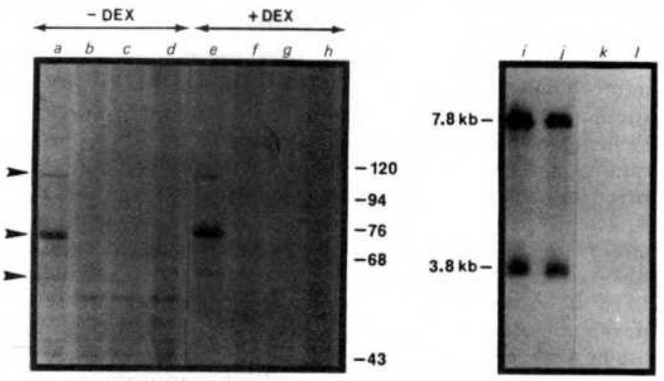

Fig. 3 Functional and biochemical analysis of MTV RNA. Total RNA, isolated from control or dexamethasone-treated (1 μM, 24 h) cultures of M1.54 and HTC by a combination of the guanidinium thiocyanate cell lysis[45] and CsCl centrifugation[46] methods, was subjected to *in vitro* translation (*a–h*) and size fractionation (*i-l*). For cell-free protein synthesis, typical 50-μl reaction mixtures contained 40 μCi ^{35}S-methionine (~1,000 Ci mmol^{-1}), 35 μl of nuclease-treated rabbit reticulocyte lysate[47,48] and 10 μg of total RNA. After 90 min at 37 °C, the incubation mixtures were added to 400 μl solubilization buffer, immunoprecipitated and fractionated in SDS gels together with molecular weight standards as in Fig. 1. Immunoprecipitations were carried out with anti-MTV serum (*a, c, e, g*) or preimmune serum (*b, d, f, h*); RNA was from uninduced M1.54 (*a, b*) and HTC (*c, d*) cells, and from hormone-induced M1.54 (*e, f*) and HTC (*g, h*) cells. Immunoprecipitations were done using 10-fold more translation products from uninduced cell RNA in order to approximately equalize levels of immunoprecipitated MTV polypeptides. Arrows denote MTV-specific *in vitro* translation products. RNA was size-fractionated by electrophoresis in formaldehyde–agarose gels[49]; 10-fold more total RNA from uninduced cells (5 μg) was analysed relative to RNA from hormone-induced cells (0.5 μg). RNA was heated to 68 °C for 5 min in 50% formamide, 2.2 M formaldehyde in running buffer (1 mM EDTA, 5 mM sodium acetate, 10 mM sodium phosphate, *p*H 7.0), then electrophoresed in 0.8% agarose gels containing 2.2 M formaldehyde in running buffer. The fractionated RNA was transferred to nitrocellulose filters in 20 × SSC (1 × SSC is 150 mM NaCl, 15 mM sodium citrate), and the filters were baked at 80 °C for 2 h *in vacuo* and incubated at 42 °C for 16 h in annealing mix [3 × SSC, 50% formamide, 50 mg ml^{-1} sheared denatured salmon sperm DNA, 5 × Denhardt's solution (1 × Denhardt's is 0.02% (w/v) each of BSA, polyvinyl pyrrolidone and Ficoll)]. The filters were then hybridized (18 h at 42 °C) with cloned MTV ^{32}P-DNA (1–3 × 10^{8} d.p.m. μg^{-1}) in annealing mix containing 10% dextran sulphate, washed three times for 20 min in 0.1 × SSC, 0.1% SDS at 50 °C and subjected to autoradiography at −70 °C. *i*, RNA from hormone-treated M1.54; *j*, RNA from uninduced M1.54; *k*, RNA from hormone-treated HTC; and *l*, RNA from uninduced HTC cells.

dexamethasone significantly alters viral phosphoprotein maturation in M1.54. The predominant phosphorylated species produced in the absence of dexamethasone is Pr74^{gag}; hormonal treatment results in the production of low levels of a second phosphorylated *gag* polyprotein, p76, and two presumptive maturation products, p35 and p24. The constitutively produced *gag* product, p55 (Fig. 1*a*, *c*), is not detectably phosphorylated. One interpretation of these data is that dexamethasone potentiates the proteolytic cleavage of one or both phosphorylated *gag* polyproteins into two smaller phosphorylated species; alternatively, the hormone might stimulate Pr74 phosphorylation to the p76 species, thereby permitting subsequent cleavage.

Two phosphorylated MTV *gag* polyproteins have also been detected in a line of mouse thymic lymphoma cells. In this case, it was found that phosphatase digestion of the larger species yielded the smaller product, implying that the larger species may reflect additional phosphorylation of the smaller[25]. We have not yet determined whether the dexamethasone-induced appearance of p76 in M1.54 cells involves a second phosphorylation of Pr74, or whether p76 is required for the apparent specific proteolytic processing of the polyprotein.

Regulation of MTV glycoprotein maturation in M1.54 cells

As covalent attachment of mannose-containing glycosylamine-linked oligosacharide side chains is one of the initial steps in cellular glycoprotein synthesis, [2-^{3}H]mannose provides a sensitive tracer for monitoring MTV glycoprotein maturation[26]. The time course of the process was examined by exposing control and hormone-induced M1.54 cultures to [2-^{3}H]mannose for 20 min, followed by additional incubation in the presence of excess unlabelled mannose. At various times during this pulse-chase, the viral proteins were immunoprecipitated with anti-MTV antiserum, fractionated by electrophoresis and visualized by fluorography.

Figure 5*a*, *c* shows that M1.54 cells initially synthesize a 74,000-M_r precursor glycoprotein, Pr74^{env}, both in the presence and absence of dexamethasone. After a 150-min chase period, the hormone-treated cells (Fig. 5*b*) appear to have processed Pr74^{env} into each of the known glycoprotein products (gp78, gp70, gp64, gp60, gp51 and gp32), while a stable form of Pr74^{env} persists. In contrast, the chased control cultures (Fig. 5*d*) produce very low levels of gp78 relative to Pr74^{env}, and yield no detectable gp70, gp60 or gp32; significantly, the uninduced cells do produce gp51 as an apparent Pr74^{env} processing product. Thus, Pr74^{env} seems to be processed in at least two distinct ways: a constitutive reaction giving a single product, and a hormone-inducible pathway resulting in a more complex mixture of species.

Localization of MTV glycoproteins was examined in separate experiments. Plasma membrane proteins were labelled by lactoperoxidase-mediated iodination of intact hormone-treated M1.54 cells; immunoprecipitation revealed that gp78, gp70, gp51 and gp32 are located at the cell surface[27]. When ^{35}S-methionine-labelled extracellular proteins were reacted with this same antiserum, we observed that hormone-treated cells secreted gp51 and gp70 (Fig. 1g, h); glycosylation of the secreted viral species was confirmed by immunoprecipitation of [2-^{3}H]mannose-labelled extracellular proteins (data not shown). In fully induced M1.54 cells, the secreted gp51 and gp70 represent ~90% of the MTV glycoproteins that accumulate over a 24-h period. Figure 1 *l*, *j* shows that uninduced cells secrete much lower levels of gp70 than gp51. Thus, gp51 maturation seems hormone-independent while the production and secretion of gp70 is glucocorticoid-regulated.

Isolation of mutants defective in MTV protein maturation

Our results are consistent with the view that dexamethasone affects a spectrum of protein maturation reactions that alter

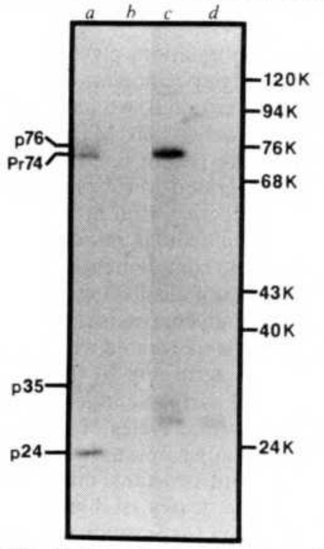

Fig. 4 Glucocorticoid-dependent maturation of MTV phosphoproteins. Dexamethasone-treated or control cultures of M1.54 were labelled with 1 mCi ^{32}P-phosphate for 12 h in phosphate-deficient medium; dexamethasone treatment was as described in Fig. 1 legend. The cell-associated MTV proteins were detergent-solubilized, immunoprecipitated and analysed as described in Fig. 1 legend; approximately 10-fold more extract from uninduced cells was used in the immunoprecipitations. *a*, Hormone-treated cells and anti-MTV serum; *b*, hormone-treated cells and preimmune serum; *c*, uninduced cells and anti-MTV serum; *d*, uninduced cells and preimmune serum. Molecular weight standards are as in Fig. 1.

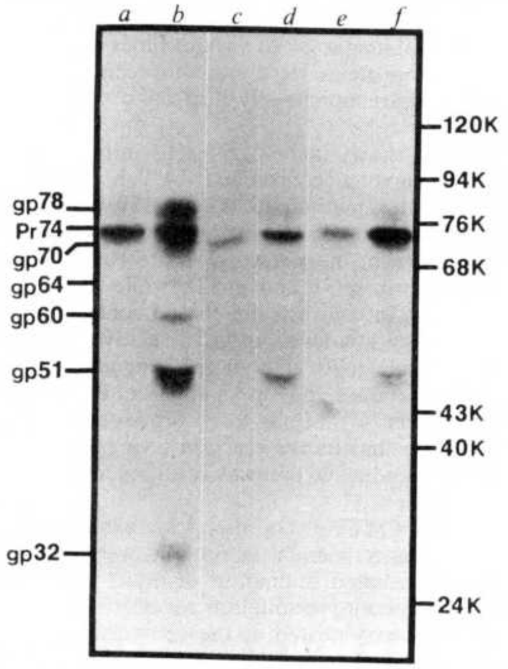

Fig. 5 MTV glycoprotein expression in M1.54 and CR4 cells. Cells were pulse-labelled at 37 °C for 20 min with 200 μCi [2-^{3}H]mannose and then incubated in the presence of 25 mM unlabelled mannose for further 150 min; hormone treatment was as described in Fig. 1 legend. Cell pellets were detergent-solubilized, immunoprecipitated and the viral glycoproteins analysed as described in Fig. 1 legend. Approximately 10-fold more material from uninduced cells was used for the immunoprecipitations. All lanes show immunoprecipitations with anti-MTV serum; parallel immunoprecipitations with preimmune serum demonstrated that all the glycoproteins detected are MTV-specific (data not shown). *a*, Pulse-labelled hormone-induced M1.54; *b*, 150 min chase of *a*; *c*, pulse-labelled uninduced M1.54; *d*, 150 min chase of *c*; *e*, pulse-labelled hormone-induced CR4; *f*, 150 min chase of *e*. Molecular weight standards are as in Fig. 1.

the efficiency of phosphorylation, glycosylation, cleavage and compartmentalization of a specific class of proteins. To examine these processes in greater detail, we have isolated mutant cell lines carrying genetic defects in MTV protein maturation. M1.54 was chosen as the parent line in our initial selections; this line carries 10 integrated MTV proviruses, thus it seems likely that mutations affecting viral protein maturation in this line will reflect lesions in cellular rather than viral sequences.

For these experiments, complement-mediated cytolysis was used to select mutants not displaying hormone-induced cell surface-associated MTV glycoproteins. M1.54 cells exposed to dexamethasone for 24 h were treated with anti-MTV antiserum and rabbit complement according to the method of Kabat *et al.*[28]; cells carrying viral surface antigens are rapidly lysed by the complement. After three cycles of selection and regrowth of surviving cells, the putative mutants were cloned and isolated.

One such complement-resistant mutant, CR4, expresses approximately wild-type levels of hormone-inducible MTV RNA[27], but is defective in the hormone-induced processing of $\mathrm{Pr74}^{\mathrm{env}}$. In the presence of dexamethasone, MTV glycoprotein processing in CR4 seems similar to the uninduced parent M1.54 cells; thus, CR4 cells essentially lack intracellular gp78, gp70 and gp32 (Fig. 1*e*, *f*; Fig. 5*e*, *f*), as well as secreted gp70 (Fig. 1*k*, *l*), whereas gp51 is produced competently and secreted. Interestingly, MTV phosphoprotein maturation seems to be unimpaired in CR4 cells (Fig. 1*e*, *f*). This genetic uncoupling of the glucocorticoid-dependent processing of phosphoproteins and glycoproteins is consistent with the view that at least two distinct protein maturation events may be hormonally regulated in HTC cells.

Conclusions and implications

Dexamethasone has striking effects on the expression of intracellular and extracellular MTV proteins in M1.54; Fig. 6 summarizes our view of the overall pattern of maturation. The dexamethasone-mediated stimulation of MTV RNA production suggests a trivial explanation for our observations: the precursor polyprotein levels may simply be increased sufficiently to allow their recognition by pre-existing maturation enzymes. However, a complement-resistant mutant of M1.54 to be described elsewhere[27] argues against this possibility, as MTV RNA and precursor protein levels are not increased by dexamethasone in that line, yet the cell surface glycoproteins are nevertheless matured.

Assuming that the processing and compartmentalization of the viral proteins is mediated by cellular components, these results suggest that glucocorticoid hormones may regulate HTC cell gene expression post-translationally. Conceivably, this mode of control by glucocorticoids could be of general significance for steroid hormonal regulation of developmental and physiological processes. It has been clearly established in a few cases that steroids affect the rate of specific mRNA transcription or turnover[4,5,29-31]. The present work now implies that these same signalling molecules can dramatically affect the expression of a gene long after it has been transcribed. In this regard, it is interesting to note that dexamethasone regulates the expression of MTV gene products in M1.54 cells both at the transcriptional and post-transcriptional levels.

Our genetic and biochemical evidence suggests that at least two post-translational maturation pathways are glucocorticoid-regulated, one controlling glycoprotein processing and compartmentalization, and another involved in phosphoprotein maturation. Recent results indicate that MTV glycoprotein maturation seems similar to that described for certain cellular proteins and for the G-protein of vesicular stomatitis virus[32]. Thus, $\mathrm{Pr74}^{\mathrm{env}}$ contains incomplete oligosaccharide side chains, as they are cleaved by endo-β-*N*-acetylglucosaminidase H (endo H)[33], while its secreted or plasma membrane-associated maturation products (gp78, gp70, gp51 and gp32) contain complete oligosaccharide side chains, as judged by their resistance to endo H digestion[33]. In addition, the cell surface forms of

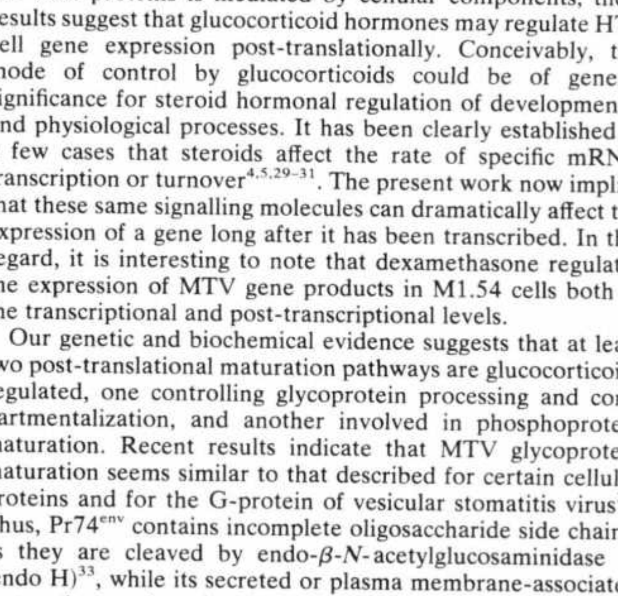

Fig. 6 Proposed pathways of MTV protein maturation in M1.54 cells. The *gag*-encoded precursor polyprotein is translated from 7.8-kilobase (kb) MTV RNA[24,50] and is differentially processed in the presence (+dex) and absence (−dex) of dexamethasone. Similarly, the *env*-encoded precursor glycopolyprotein is translated from 3.8-kb MTV RNA[24,46] and then processed in a hormone-dependent manner. s, Secreted glycoproteins; m, plasma membrane-associated species. Several larger precursors (Pr200, Pr160, Pr105) are also detected (see Fig. 1); these minor species are not shown in this diagram.

gp78, gp70 and gp32 are modified by the attachment of palmitic acid (G.L.F., unpublished result).

Clearly, there are many potential targets for hormone regulation of protein maturation. For example, glycosyltransferases, glycosidases, proteases or protein kinases that recognize the viral proteins as substrates could be hormone-induced at transcriptional or post-transcriptional levels. In fact, steroid hormones have been reported to alter the apparent activities or concentrations of such enzymes, but specific substrates and direct molecular and genetic approaches were not available in these cases[34–38]. Regulation of protein processing activities could affect not only the 'targeting' of proteins to particular locations, but might also change the stability of the polyprotein precursors. Another possibility is that dexamethasone alters the expression of 'compartmentalization factors' analogous to those involved in intracellular membrane localization of β-glucuronidase in kidney epithelia[39], or to β_2-microglobulin-mediated membrane insertion of histocompatibility locus antigens[40].

In any case, it is apparent that glucocorticoids are capable of regulating post-translational aspects of gene expression. These hormonal effects were detected in the present studies by exploiting MTV gene products as specific probes within HTC cells. As MTV appears to infect cultured cells from a broad range of species and tissue types, it may be possible to use these genes to test the generality of these protein maturation regulatory phenomena. Recently, we have detected the dexamethasone-dependent production of an MTV phosphopolyprotein in GR cells, a virion-producing mouse mammary carcinoma cell line (G.L.F., unpublished result). Given these sensitive molecular probes, it may be possible to elucidate the mechanisms by which glucocorticoids affect protein maturation.

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Recombination between dispersed serine tRNA genes in *Schizosaccharomyces pombe*

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During meiosis in fission yeast, transfer of genetic information occurs between tRNA genes of related sequence even when they are located on different chromosomes. This process of intergenic conversion has been analysed with the help of nonsense suppressors derived from three unlinked serine tRNA genes. In their wild-type form two of the genes code for $\mathrm{tRNA}^{\mathrm{Ser}}_{\mathrm{UCA}}$, *the third for* $\mathrm{tRNA}^{\mathrm{Ser}}_{\mathrm{UCG}}$. *We demonstrate the transfer of anticodon and adjacent intron sequences between two tRNA genes coding for different serine isoacceptors.*

THE first gene families studied in detail were the tandemly arranged ribosomal RNA genes. To explain the striking sequence conservation between individual copies in a given species, several rectification models have been proposed[1]. One of these mechanisms, unequal crossing-over, has been documented experimentally for the rRNA genes in *Saccharomyces cerevisiae*[2,3] and *Salmonella typhimurium*[4]. Meanwhile, it has been found that rectification mechanisms must also be responsible for the concerted evolution[5] observed in other gene families which are not necessarily arranged in tandem, such as the repeated globin genes[5,6] and immunoglobulin genes[7]. Further examples include separated blocks of tandemly repeated genes that are located on non-homologous chromosomes, that is, rRNA genes[8,9] and repetitive sequences of unknown function[10]. Finally, concerted evolution has also been observed among the completely dispersed 5S rRNA genes in *Neurospora*

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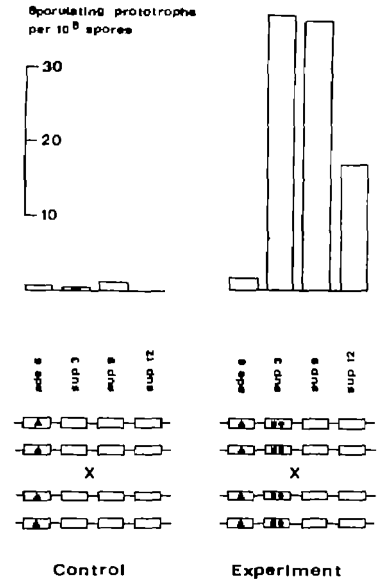

Fig. 1 Frequencies of prototrophs in experimental selfings and controls. The contrasting results obtained from the control cross no. 13 and the experimental cross no. 15 are shown (see Table 1). The gene symbols apply to both the upper and lower parts of the figure. The lower part depicts the crosses between diploid parents. This schematic representation does not reflect the known linkage relationships between the genes concerned. In both cases all *ade6* loci were occupied by a suppressible nonsense allele (*ade6-704*, ▲). The only difference between the two crosses concerns the *sup3* genes: in the control all were wild type; the experimental cross was homozygous for the *sup3-UGA,r36* allele, combining suppressor active anticodon information (■) with a secondary inactivating lesion (*r36*, ●). All other suppressor loci were occupied by wild-type alleles in both crosses. The upper part gives the frequency of sporulating prototrophic revertants owing their prototrophy to a reversion of the *ade6* mutant gene or to an active suppressor as indicated. Overall frequencies would be 50% higher because only sporulating revertants heterozygous for mating type have been analysed. We assume that the pattern of events is the same for the sporulating (2/3) and the non-sporulating (1/3) population, but under any assumption the marked increase of *sup3-UGA*, *sup9-UGA* and *sup12-UGA* events in the experimental situation, compared with the control, would persist.

crassa[11]. Molecular mechanisms proposed to contribute to the concerted evolution not only of tandemly arranged but also of dispersed repeated genes include gene transposition[11] and intergenic conversion[6,7,9,12,13].

Intergenic conversion implies an information transfer between nonallelic genes of related nucleotide sequence by a mechanism analogous to that proposed for recombination of allelic genes[14]. Within a family of directly repeated genes, intergenic conversion without associated crossing-over does not lead to copy-number variation. Therefore, gene duplications and deletions which are the consequence of models based solely on unequal crossing-over do not necessarily occur during concerted evolution. In the case of dispersed repeated genes, intergenic conversion may well be the main mechanism permitting co-evolution. That intergenic conversion really occurs in mitosis and meiosis has been demonstrated in *S. cerevisiae* for pairs of mutationally inactivated protein structural genes[15–17]. In these studies a second copy of a given gene was integrated at various sites in the genome by transformation and in those cases in which the two genes carried different mutations, fully active genes were obtained by recombination. The two cytochrome *c* genes in *S. cerevisiae* are examples of naturally occurring genes with related sequences mapping on different chromosomes. In this case, DNA sequencing of revertants of several mutations revealed the import of blocks of wild-type sequence from the corresponding non-identical gene[18].

Transfer RNA genes are widely dispersed in the genomes of most organisms. Nevertheless, multiple genes coding for a specific isoacceptor very often maintain one common nucleotide sequence in the segments that code for the mature tRNA. We previously reported genetic evidence that intergenic conversion occurs between dispersed serine tRNA genes in *Schizosaccharomyces pombe*[19]. Here we present further genetic and physical data indicating that information transfer occurs spontaneously during meiosis between three unlinked serine tRNA genes that code in their wild-type form for two different serine isoacceptor tRNAs. We show that the different anticodons and also adjacent diverging sequences of the introns may be exchanged between these tRNA genes.

The genes *sup3* on chromosome I and *sup9* on chromosome III are the only ones that in their wild-type form encode the UCA reading isoacceptor with the anticodon U*GA (where U* is a modified uridine)[20]. Both have been sequenced recently (ref. 21 and D. Söll, personal communication) and are also available as opal (UGA) suppressor alleles coding for tRNAs with the anticodon U*CA[22]. The single gene *sup12* on chromosome I codes in its wild-type form for the UCG reading serine tRNA with the anticodon CGA and has been sequenced earlier[23]. All three genes are organized in a unique way (Fig. 4): the serine tRNA coding sequences are identical with the exception of one differing base pair corresponding to the tip of the tRNA extra loop and of the obvious differences between the anticodon sequences. The genes *sup3* and *sup9* ($\text{tRNA}^{\text{Ser}}_{\text{UCA}}$) carry identical introns of 15 base pairs (bp) which differ at six positions from the 16-bp intron of *sup12* ($\text{tRNA}^{\text{Ser}}_{\text{UCG}}$). On the 3′ side of each of these serine tRNA genes follows a completely conserved DNA sequence comprising a spacer of 7 bp and a gene coding for initiator tRNA^{Met}. Thus, the region of sequence homology between the three DNA segments is ~200 nucleotides long including a few partially conserved base pairs in the flanking regions.

Informational transfer between non-linked serine tRNA genes is documented genetically by comparing two types of crosses. In type I, the control, the two parents are identical, except for mating-type, and carry a nonsense mutation in a

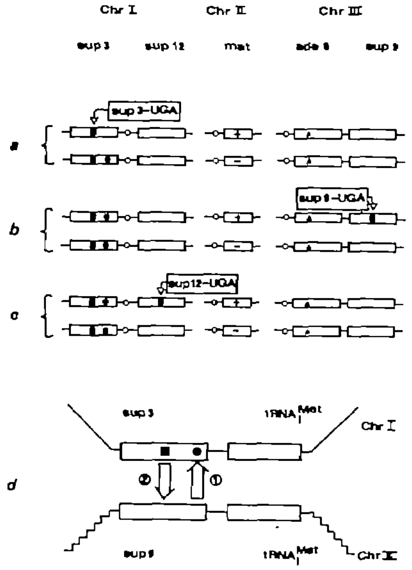

Fig. 2 Import and export of genetic information by intergenic conversion. The genotypes of the predominant classes of prototrophs obtained from cross no. 15 are depicted. *sup3* and *sup12* are on chromosome I, yet genetically not closely linked with each other. *mat* refers to the mating-type locus on chromosome II. *ade6* and *sup9* are located on chromosome III[26]. Empty rectangles represent wild-type genes, symbols as in Fig. 1. *a–c*, Prototrophic diploids owing their adenine independence to one copy of the dominant and efficient suppressors *sup3-UGA*, *sup9-UGA* and *sup12-UGA*, respectively. *d*, A schematic representation of the mechanism thought to generate the vast majority of these prototrophic revertants. For example, the wild-type gene *sup9*$^+$ may interact during meiosis with the related partner *sup3-UGA,r36*. A single-site conversion replacing the inactivating *r36* site, but not the anticodon site, of the doubly marked *sup3* gene by a corresponding wild-type segment from *sup9*$^+$ leads to an active *sup3-UGA* allele (1). On the other hand, transfer of the suppressor active anticodon sequence, divorced from *r36*, to the wild-type gene *sup9*$^+$ will generate a *sup9-UGA* suppressor (2). As in conversion involving allelic genes the donating partner is found to emerge unchanged from the interaction. Entirely analogous events are postulated to occur also between *sup3-UGA,r36* and *sup12*$^+$

biosynthetic gene. They are thus auxotrophic. In type II, the experimental situation, the parents carry in addition an identical inactive suppressor allele at corresponding loci. These alleles have suppressor-active anticodon information (obtained by mutation in a first step) but are prevented from suppressing the background nonsense mutation due to a secondary intragenic lesion (obtained in a second mutational step). They are thus also auxotrophic. In both cases prototrophic revertants can be obtained by plating progeny spores on media lacking the growth factor whose biosynthesis is prevented by the nonsense mutation. Type I gives few revertants which result from reversion of the nonsense mutation and from forward mutations at suppressor loci in comparable frequencies. This represents the mutational background. Type II shows a dramatic increase in reversion frequency. The surplus events occur both in the suppressor gene that was originally doubly marked in the parents of the cross and in the two remaining serine tRNA genes which were originally present in wild-type form. Mutation cannot explain this result. Also, recombination between the doubly-marked suppressor genes of the parents does not lead to an active suppressor because these genes are identical. Instead, we propose that non-allelic members of this gene family may interact and exchange information in a process analogous to gene conversion between allelic genes. Non-reciprocal information transfer may occur in either direction when the doubly-marked gene interacts with a wild-type member of the family: replacement of the secondary lesion by wild-type information leads to an active suppressor in the former, transfer of the suppressor-active anticodon information into the wild-type gene leads to an active suppressor in the latter. Physical analysis has corroborated this interpretation, as restriction mapping and DNA sequencing show that genes obtained by this process of intergenic conversion may differ from the original copy at several non-adjacent sites, reflecting the transfer of DNA segments.

Two known suppressor tRNA genes import information from non-allelic genes

To detect rare intergenic convertants an experimental system is needed that allows discrimination against events caused by recombination between allelic genes. This we have achieved by carrying out homozygous crosses, that is, selfings of strains carrying inactivated suppressors.

A first series of experiments involved the selfing of auxotrophic haploid strains and the analysis of the rare prototrophic revertants obtained among the progeny spores. The results are summarized in the first part of Table 1 (expts 1–12). Strains carrying either the UGA nonsense mutation *ade6-704* or *ade1-40* in combination with wild-type genes at all suppressor loci served as controls (expts 1 and 8). The experimental strains carried, on the background of one or the other of these nonsense mutations, inactive suppressor alleles combining suppressor active anticodon information with a secondary inactivating lesion[24] (*sup3-UGA,rX*: expts 2–7; *sup9-UGA,rX*: expts 9–12). It is obvious that the reversion frequency is much higher in the experimental selfings than in the controls. Furthermore, the prototrophy of the revertants obtained from the experi-

Table 1 Frequency and nature of prototrophic revertants from selfings

Expt. no.	Ploidy of strains	Suppressor allele involved	No. of independent expts	Prototrophic revertant colonies observed	Total spores analysed ($\times 10^8$)	Reversion frequency ($\times 10^{-8}$)	Nature of prototrophic revertants: *ade*$^+$	*sup3-UGA*	*sup9-UGA*	*sup12-UGA*	*sup8-UGA*	*sup10-UGA*	Others	Total revertants analysed
1	*n*	*sup3*$^+$ (UCA)	3	14	9.0	2		10	3		1			14
2	*n*	*sup3-UGA,r8*	3	319	10.1	32		76					3	79
3	*n*	*sup3-UGA,r10*	3	2474	12.0	206		73					1	74
4	*n*	*sup3-UGA,r15*	3	158	11.7	14		63					15	78
5	*n*	*sup3-UGA,r30*	3	955	9.9	96		58			1		4	63
6	*n*	*sup3-UGA,r36*	3	914	10.2	90		80						80
7	*n*	*sup3-UGA,r40*	3	819	15.6	53		75					4	79
8	*n*	*sup9*$^+$ (UCA)	3	17	5.3	3	3	5	9					17
9	*n*	*sup9-UGA,r49*	3	1950	16.9	115			58		1		5	64
10	*n*	*sup9-UGA,r68*	3	987	15.3	65			68				2	70
11	*n*	*sup9-UGA,r101*	3	602	13.5	45			73		1		4	78
12	*n*	*sup9-UGA,r104*	3	713	6.5	110			64		1		2	67
13	2*n*	*sup3*$^+$ (UCA)	6	10	2.7	4	2	1	3			1		7
14	2*n*	*sup3-UGA,r8*	4	38	1.5	25		10	4	2		1		17
15	2*n*	*sup3-UGA,r36*	4	198	1.3	152	2	48	47	22				119

Strains 1–7 are of general constitution h^{90} *ade6-704 sup3* and strains 8–12 of constitution h^{90} *ade1-40 sup9* with the particular suppressor alleles indicated. They are haploid and homothallic, that is, self-sporulating. In sporulating conditions they produce diploid zygotes leading on meiosis to asci containing four haploid and homothallic spores. Thus, this procedure is equivalent to crossing two heterothallic strains differing only at the mating-type locus (h^+ and h^-). *ade6-704* and *ade1-40* are both suppressible UGA nonsense mutations which lead, when not suppressed, to an adenine requirement and, in the case of *ade6-704*, to the formation of a characteristic red pigment in colonies grown on media with low adenine concentrations such as yeast extract agar. Crosses 13–15 involve two diploid parents each and are of the general type (h^+/h^+ *ade6-704/ade6-704 sup3/sup3*)×(h^-/h^- *ade6-704/ade6-704 sup3/sup3*) with particular *sup3* alleles indicated. The two parents of any of these three crosses are different only with respect to the mating-type locus. Such matings result in tetraploid zygotes which after meiosis produce asci with four diploid spores. Two-thirds of these spores are of heterozygous mating-type constitution (h^+/h^-) and give rise to diploid clones, which in turn will sporulate in appropriate conditions producing haploid progeny. They are directly amenable to genetic testing, in contrast to the last third which is stable and of constitution h^+/h^+ and h^-/h^-, respectively. The suppressor alleles involved in these selfings are either wild-type or double-mutant alleles combining suppressor active anticodon information with a secondary inactivating lesion elsewhere in the suppressor gene[24]. Selfings 1, 8 and 13 represent controls and are alike in the sense that only wild-type alleles are involved at all suppressor loci concerned. Selfings were done on malt extract agar slants supplemented with adenine (8 days, 25 °C). The sporulated cultures were suspended in water, treated overnight with snail digestive juice to inactivate remaining vegetative cells, washed and resuspended in 5 ml water. 4 ml of each suspension were distributed on 20 minimal agar plates in order to select prototrophic revertants (selfings 1–12: 7 days, 30 °C; selfings 13–15: 9 days, 25 °C). Aliquots of appropriate dilutions were spread on yeast extract agar for total spore counts. The number of independent repeats of a particular selfing is indicated Pooled data are given as no cases of extreme fluctuation were observed A sample of the revertants was analysed to determine the factor responsible for prototrophy. In the case of selfings 2–7 and 9–12 some 70 randomly chosen revertants were analysed per selfing. These were taken in approximately equal numbers from the three repeats. The sample in selfings 13–15 consisted of all sporulating revertants obtained. The nature of these revertants was assessed in crosses with a set of five haploid tester strains: a wild-type strain and four mutant strains each of which carried one of four efficient suppressors on the background of *ade6-704*. The haploid revertants from selfings 1–12 could be directly crossed to the testers. From the sporulating diploid revertants of selfings 13–15, 8 tetrads were analysed per revertant strain. Class I isolates produced two prototrophic and two adenine-dependent segregants per tetrad. One prototrophic haploid segregant was used for testing. Class II isolates were observed only in selfings 14 and 15 and gave two lethal and two auxotrophic segregants per tetrad. They could be subdivided in classes IIA and IIB as the lethality in the former class was due to a mutant *sup3* background (*sup3-UGA,rX*) and haplo-vital prototrophic derivatives, amenable to testing, could be obtained on replacement of *sup3-UGA,rX* by *sup3*$^+$. The lethality in the latter class could not be removed by this procedure. Crosses between the revertants and the five testers were performed and haploid progeny spores plated on yeast extract agar. If the progeny colonies of a particular test cross were only white, it was concluded that the strain under study and the tester carried the same determinant responsible for prototrophy (the same suppressors in the case of suppressor testers, an *ade*$^+$ gene in the case of the wild-type tester). This positive indication of allelism was complemented by analysing at least one of the four remaining crosses with the same revertant. Apart from the special situation described below, such crosses gave white and red colonies, the latter being suppressor free *ade6-704* recombinants. In the case of revertants originating from selfings involving *ade1-40* (nos 8–12) progeny from crosses with the wild-type tester had to be replica plated onto minimal agar to check for auxotrophic recombinants since the red pigment marker (*ade6-704*) was absent in both strains of such crosses.

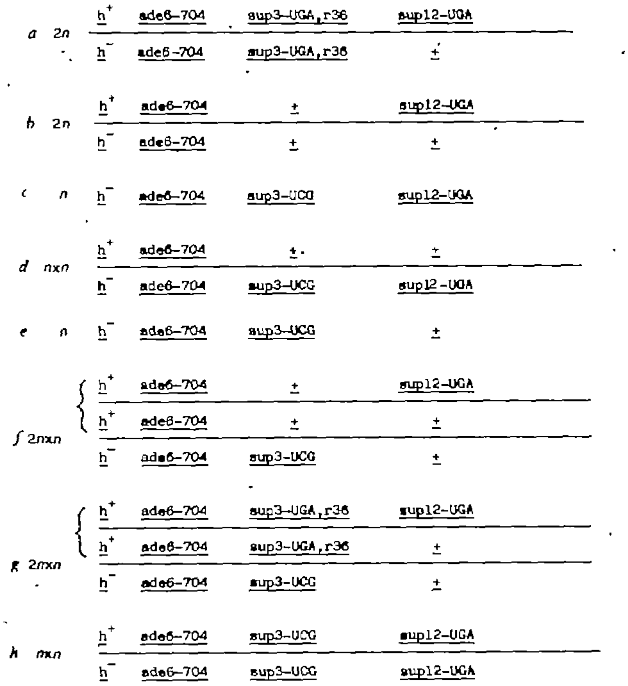
a 2n
h+ ade6-704 sup3-UGA,r36 sup12-UGA
h− ade6-704 sup3-UGA,r36 +
b 2n
h+ ade6-704 + sup12-UGA
h− ade6-704 + +
c n
h− ade6-704 sup3-UCG sup12-UGA
d nxn
h+ ade6-704 + +
h− ade6-704 sup3-UCG sup12-UGA
e n
h− ade6-704 sup3-UCG +
f 2nxn
h+ ade6-704 + sup12-UGA
h+ ade6-704 + +
h− ade6-704 sup3-UCG +
g 2nxn
h+ ade6-704 sup3-UGA,r36 sup12-UGA
h+ ade6-704 sup3-UGA,r36 +
h− ade6-704 sup3-UCG +
h nxn
h+ ade6-704 sup3-UCG sup12-UGA
h− ade6-704 sup3-UCG sup12-UGA

Fig. 3 Isolation of haplo-vital *sup12-UGA* strains by the generation of a *sup3-UCG* allele. The crosses involved and the strains used are identified with letters *a–h*. The ploidy of the strains is indicated by *n* (haploid) and 2*n* (diploid). For *f*, *g* and *h* crosses with the reciprocal mating-type configuration were also performed, depending on the nature of the isolate to be tested. For further explanations see text.

mental selfings was due to an active suppressor allelic with the doubly-marked input allele in the vast majority of cases (that is, *sup3-UGA,rX* strains produced predominantly *sup3-UGA* revertants and *sup9-UGA,rX* strains produced mostly *sup9-UGA* prototrophs). This contrasts with the controls which gave back mutations at the *ade* loci and forward mutations at the suppressor loci with varying but comparable frequencies. The revertants designated 'others' in Table 1 have not been analysed further. They might comprise weak omnipotent suppressors or allosuppressors[25].

On the basis of mutation alone one would have to postulate that the inactivating *r* sites would have an unusually high probability of being changed back to the original wild-type state, thus restoring suppressor activity. This explanation is very unlikely. We therefore rather assume that recombination is responsible for the observed phenomenon. As identical alleles of the marked suppressor gene are involved in selfings, no recombinants are expected to occur following their interaction. We postulate that non-allelic but related wild-type genes (that is, other $\mathrm{tRNA}^{\mathrm{Ser}}$ genes) interact with the doubly marked gene. According to this interpretation, gene segments carrying the inactivating *r* sites but not the anticodon sequence are replaced by the corresponding sequences of the wild-type partners in a unidirectional process of gene conversion[14].

Besides the observed import of information into the marked suppressor gene, export of the suppressor active anticodon sequence into related wild-type genes is also expected to occur. Prime candidates to act as acceptors are the *sup9*$^+$ gene in *sup3-UGA,rX* selfings and the *sup3*$^+$ gene in *sup9-UGA,rX* selfings. If such transfers occur non-reciprocally, however, the haploid progeny will be lethal because both $\mathrm{tRNA}^{\mathrm{Ser}}_{\mathrm{UCA}}$ genes will be of mutant constitution, thus depriving the cell of functional $\mathrm{tRNA}^{\mathrm{Ser}}_{\mathrm{UCA}}$ (for example, *sup3-UGA,rX sup9-UGA*)[20]. That no-*sup9-UGA* revertants are observed in selfings involving *sup3-UGA,rX* and vice versa is thus expected and rests on the special situation in *S. pombe* that *sup3*$^+$ and *sup9*$^+$ are the only two genes producing functional $\mathrm{tRNA}^{\mathrm{Ser}}_{\mathrm{UCA}}$. The experimental system has therefore to be modified so as to demonstrate export of suppressor active anticodon information into companion wild-type genes.

Export of information generates a novel haplo-lethal suppressor

To determine whether the postulated export of suppressor active anticodon information does occur, we analysed selfings of diploid strains. The results are given in the lower part of Table 1 (expts 13–15) and in Fig. 1. Our interpretation is outlined in Fig. 2. Again, as in the case of haploid strains, the reversion frequency was clearly elevated in selfings involving inactivated suppressor genes as compared with the control. The selfing involving the *sup3-UGA,r36* allele is particularly instructive. Not only did we find prototrophic diploids carrying active *sup3-UGA* alleles, but also strains carrying *sup9-UGA* or a new suppressor, *sup12-UGA*, in significant excess over the control (Fig. 1).

The *sup3-UGA* bearers are explained as in the case of haploid selfings. The increased occurrence of *sup9-UGA* events is taken as evidence for genetic export of the suppressor active anticodon sequence from *sup3-UGA,rX* to *sup9*$^+$, again via gene conversion. Note that in this case the two interacting partners are located on different chromosomes (Fig. 2). These strains can survive as diploids, in contrast to the haploid situation, because they harbour one unmutated $\mathrm{tRNA}^{\mathrm{Ser}}_{\mathrm{UCA}}$ gene (*sup9*$^+$).

Evidently, a second gene can accept suppressor active anticodon information by the same mechanism, namely *sup12*$^+$ (UCG). The original constitution of these revertants is depicted in Fig. 2*c*. On sporulation, each tetrad gave two lethal and two auxotrophic spores. Thus, *sup12-UGA* is a recessive lethal suppressor. That it is a suppressor in its own right and not an allosuppressor acting in conjunction with *sup3-UGA,rX* was demonstrated by replacing the doubly-marked *sup3* background (for example, *sup3-UGA,r36*) by a homozygous wild-type background (*sup3*$^+$). Such diploids of constitution h^+ *ade6-704 sup12-UGA*/h^- *ade6-704 sup12*$^+$ are still prototrophic and produce two lethal and two auxotrophic segregants per tetrad on sporulation. In addition, *sup12* does not show genetic linkage with either *sup3* or *sup9* (data not shown).

The *sup12* gene, which had not been identified genetically before, is identical with the $\mathrm{tRNA}^{\mathrm{Ser}}_{\mathrm{UCG}}$ gene whose sequence has been published[23] (see below). As *sup3*$^+$ and the $\mathrm{tRNA}^{\mathrm{Ser}}_{\mathrm{UCG}}$ gene differ only by two base pairs in their coding regions, it is likely that they can interact as discussed above. Because *sup12-UGA* is haplo-lethal, we conclude that no substitute exists for the product of the $\mathrm{tRNA}^{\mathrm{Ser}}_{\mathrm{UCG}}$ gene. In addition, it is interesting that the change of *sup12*$^+$ (UCG) to *sup12-UGA* requires 2 bp changes in the anticodon sequence. This should easily occur via gene conversion but only very rarely by spontaneous mutation.

Haplo-vital *sup12-UGA* strains can be derived by further intergenic exchanges

As genetic analysis with a haplo-lethal suppressor is tedious, we sought a way to change one of the genes coding for UCA reading tRNA to an allele producing a UCG reading tRNA. The procedure to obtain haplo-vital *sup12-UGA* isolates is outlined in Fig. 3. The experiment was started with one of the original *sup12-UGA* diploids derived from selfing 15 (Table 1) and is depicted in Fig. 3*a*. A derivative of (*a*) homozygous for one of the mating-type alleles was crossed to an *ade6-704/ade6-704* diploid homozygous for the opposite mating-type allele. From this cross segregant (*b*) was obtained. On sporulation, vital progeny were dark red exclusively (*ade6-704 sup3*$^+$), in contrast to the meiotic progeny of (*a*) which are all pink, the particular *sup3* allele involved being responsible for this colour difference (*ade6-704 sup3-UGA,r36*). The vast majority of haploid spores from (*b*) will not grow on minimal agar: 50% are lethal due to *sup12-UGA*, 50% are adenine dependent. Nevertheless, two types of haploid prototrophs might rarely occur via mutation or information transfer: (1)

Active suppressors at the *sup3* or *sup9* locus, if they inherit at the same time *sup12*$^+$. They are normal suppressors and will not generate lethal progeny in crosses with standard partner strains. (2) Strains with a mutant anticodon information at *sup3* or *sup9* resulting in the production of $tRNA^{Ser}_{UCG}$ rather than $tRNA^{Ser}_{UCA}$ (for example, strain *c*, Fig. 3). If they carry *sup12-UGA* at the same time, such strains are haplo-vital prototrophs; lethality resulting from mutation of the only naturally occurring $tRNA^{Ser}_{UCG}$ producer (*sup12*) has now been by-passed. For instance, *sup3-UCG* will produce $tRNA^{Ser}_{UCG}$, *sup9*$^+$ will form $tRNA^{Ser}_{UCA}$ and *sup12-UGA* suppressor-active $tRNA^{Ser}_{UGA}$. In crosses with standard partners, lethal progeny are expected to be produced due to the separation of *sup3-UCG* from *sup12-UGA*.

Among ~3×10^7 spores of strain (*b*), 11 haplo-vital prototrophs were obtained. Ten of them did not segregate lethal progeny in crosses with a standard partner and were discarded. The remaining one of presumed constitution (*c*) was crossed to *h*$^+$ *ade6-704* (*d*). The following tetrads were observed: 9 parental ditypes (2 red and 2 white colonies per tetrad), 17 tetratypes (2 red, 1 white, 1 lethal) and 5 non-parental ditypes (2 red and 2 lethal). The same strain (*c*) was also crossed to *h*$^+$ *ade6-704 sup3-UGA* and *h*$^+$ *ade6-704 sup9-UGA*. These crosses gave results compatible with two and three independent factors segregating, respectively, thus indicating that the derived strain carries a *sup3-UCG* rather than a *sup9-UCG* allele, as shown in (*c*). Physical evidence that the *sup3-UCG* allele originated from intergenic conversion and not from mutation is given below.

Red strains from non-parental ditype tetrads of cross (*d*) should be of constitution (*e*). Although phenotypically not distinguishable from normal red *ade6-704* strains, they are predicted to carry the mutant *sup3* allele *sup3-UCG*. This 'helper' strain was crossed to mating-type homozygous derivatives of all 22 *sup12-UGA* diploids obtained from selfing 15 (Table 1). These triploid crosses are shown diagrammatically in (*f*) and (*g*). Some of the diploid parents used carried the original *sup3-UGA,r36* background (*g*), others the *sup3*$^+$ background (*f*). In all cases haploid prototrophic recombinants were easily obtained. In contrast, this did not occur if a normal *ade6-704 sup3*$^+$ strain was the haploid parent in such triploid crosses. All these haploid prototrophs were involved in crosses with each other (cross type (*h*)). In all cases the vast majority of progeny colonies was white. This indicates that the determinants responsible for prototrophy in the original diploids are indeed allelic (*sup12-UGA*).

Having obtained haplo-vital *sup12-UGA* strains, we proceeded to determine the chromosomal location of this suppressor (data not shown). Based on the analysis of 94 tetrads of a cross homozygous for *ade6-704* and *sup3-UCG* and heterozygous for *ura1*, *pro1* and *sup12-UGA*, we inferred the arrangement *ura1*–23 centimorgans–*pro1*–9cM–*sup12* on chromosome I[26].

Physical evidence for intergenic conversion

By restriction analysis of DNA from the relevant strains and by filter hybridization experiments according to Southern[27] we obtained evidence for the identity of the *sup12-UGA* suppressor allele with the known $tRNA^{Ser}_{UCG}$ gene. The data also ndicate that non-adjacent base pairs have been substituted in *sup3-UCG* in comparison with *sup3*$^+$ (Fig. 4). In addition, *sup3-UCG* has been sequenced (Fig. 5).

Genomic blots give complex hybridization patterns, because one is dealing with a family of cross-hybridizing genes. In addition to the well characterized *sup3*, *sup9* and *sup12* genes there is a further related sequence present in the genome (data not shown). Figure 4*a* gives a simplified restriction map of the chromosomal region with the $tRNA^{Ser}_{UCG}$ gene and the sequence of its non-coding strand comprising the anticodon and the 5′ part of the intron[23]. The sequence of the corresponding region of *sup3-UGA* is also given[21].

The phenotypic effect of *sup12-UGA* concerning suppression is identical with that of the other opal suppressors inserting serine (*sup3-UGA*) or leucine (*sup8-UGA*) which are known to code for suppressor tRNAs with the anticodon U*CA[28,29]. Thus, *sup12-UGA* most probably carries the anticodon sequence TCA which abolishes the two restriction sites present at this location in *sup12*$^+$ (Fig. 4*a*). In fact, a new and longer *Eco*RI restriction fragment is found in DNA of *sup12-UGA*/*sup12*$^+$ diploids. In the haplo-vital *sup3-UCG sup12-*

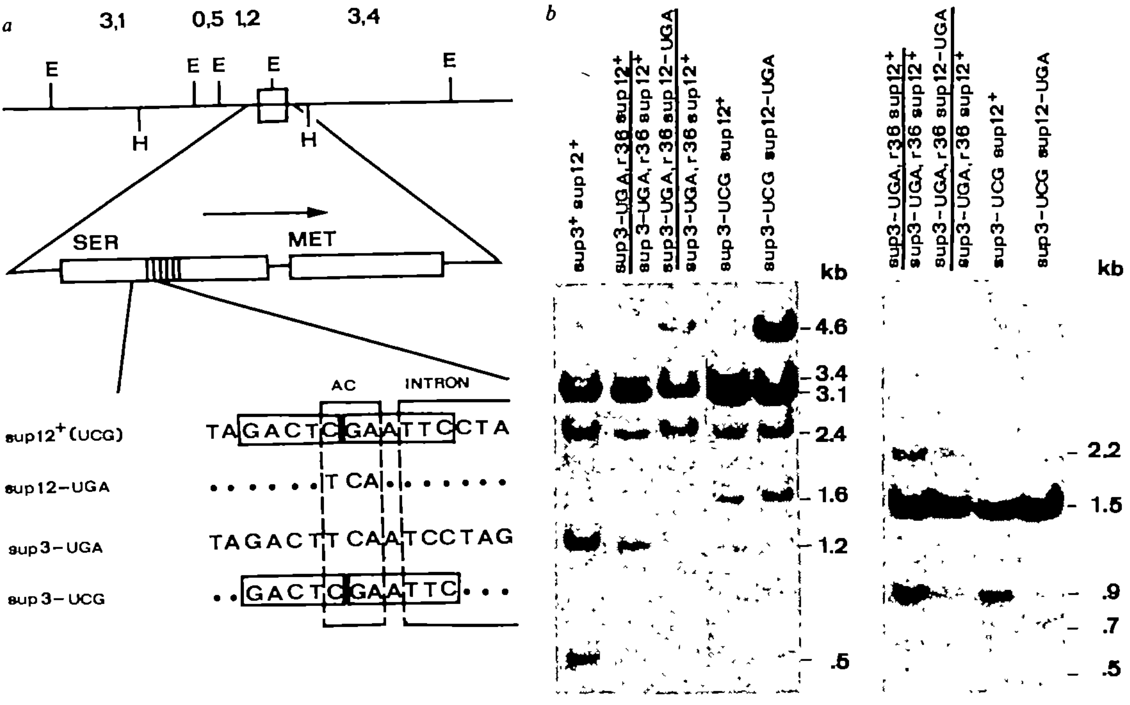

Fig. 4 Physical evidence for intergenic conversion. *a*. The chromosomal organization of the *sup12* locus and the relevant sequences of the anticodon regions of *sup12*$^+$ and *sup3-UGA* are represented[21,23] The *Eco*RI restriction sites (E) and the size of the resulting fragments in kilobases are indicated. The two *Hin*dIII sites (H) delimit the cloned fragment present in the chimaeric plasmid pYM118 that carries the $tRNA^{Ser}_{UCO}$–$tRNA^{Met}_{i}$ genes[23]. In the blow up the tRNA genes are shown as boxes. The shaded area signifies the intron and the arrow the direction of transcription Below, excerpts of the published sequences of *sup12*$^+$ (ref. 23) and *sup3-UGA*[21] are given as well as the deduced sequences of the two intergenic convertants analysed. The anticodons (AC) and the 5′ border of the introns are marked with broken lines The relevant restriction sites recognized by the *Eco*RI (GAATTC) and *Hin*fI (GANTC) endonucleases are boxed. *b*, On the left hybridization patterns according to Southern[27] are shown that were obtained after digestion of genomic DNA with *Eco*RI. The corresponding results obtained with *Hin*fI digests are given on the right. The hybridization probe consisted in the nick-translated *Hin*dIII fragment from the recombinant plasmid pYM118[23]. Above each lane the genotype of the haploid or diploid strain is given that served as source of the DNA The novel *Eco*RI fragment of 4.6 kb length is present in strains carrying *sup12-UGA*, while the new fragment of 1.6 kb length correlates with *sup3-UCG*. In strains with the *sup3-UCG* allele the 2.2 kb *Hin*fI fragment is missing and a new 0.7 kb fragment appears. For more details see the text.

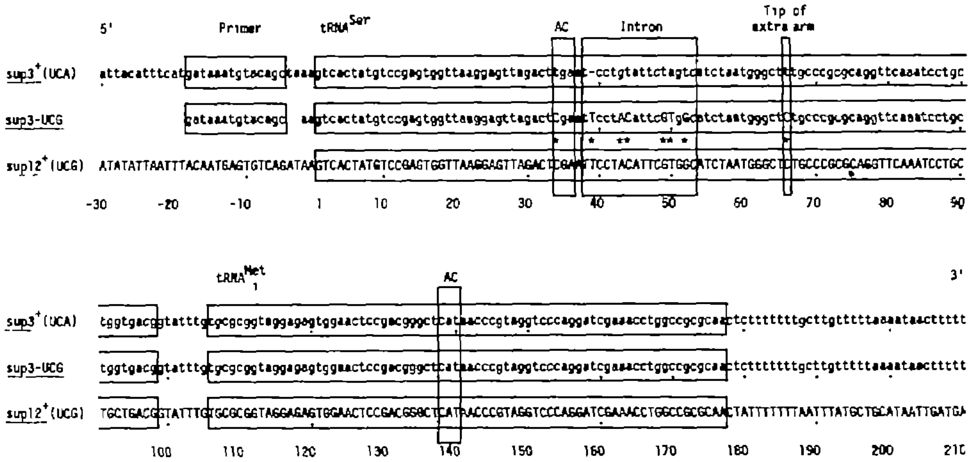
5'
Primer
tRNA^Ser
AC
Intron
Tip of extra arm
sup3+(UCA) attacatttcatgataaatgtacagctaaagtcactatgtccgagtggttaaggagttagacttgaat-cctgtattctagtcatctaatgggctttgcccgcgcaggttcaaatcctgc
sup3-UCG gataaatgtacagc aagtcactatgtccgagtggttaaggagttagactCgaatTcctACattcGTgGcatctaatgggctCtgcccgcgcaggttcaaatcctgc
* * ** ** * *
sup12+(UCG) ATATATTAATTTACAATGAGTGTCAGATAAGTCACTATGTCCGAGTGGTTAAGGAGTTAGACTCGAAATTCCTACATTCGTGGCATCTAATGGGCTCTGCCCGCGCAGGTTCAAATCCTGC
-30 -20 -10 1 10 20 30 40 50 60 70 80 90
tRNA^Met_1
AC
3'
sup3+(UCA) tggtgacggtatttgtgcgcggtaggagagtggaactccgacgggctcataacccgtaggtcccaggatcgaaacctggccgcgcaactctttttttgcttgtttttaaaataactttt
sup3-UCG tggtgacggtatttgtgcgcggtaggagagtggaactccgacgggctcataacccgtaggtcccaggatcgaaacctggccgcgcaactctttttttgcttgtttttaaaataactttt
sup12+(UCG) TGCTGACGGTATTTGTGCGCGGTAGGAGAGTGGAACTCCGACGGGCTCATAACCCGTAGGTCCCAGGATCGAAACCTGGCCGCGCAACTATTTTTTTAATTTATGCTGCATAATTGATGA
100 110 120 130 140 150 160 170 180 190 200 210

Fig. 5 Nucleotide sequence of the intergenic convertant *sup3-UCG*. The noncoding strands of the tRNA genes *sup3⁺* (small letters) and *sup12⁺* (capitals) and their flanking sequences were taken from refs 21 and 23. Between them the sequence of *sup3-UCG* is given. It differs from *sup3⁺* at all of the eight positions (capitals) that vary between *sup3⁺* and *sup12⁺* (asterisks). Thus, the conversion event includes a block of at least 33 nucleotides. No change was observed between *sup3-UCG* and *sup3⁺* in the 3′-flanking region for the first 40 nucleotides downstream of the tRNA$_1^{Met}$ gene. In the 5′-flanking region at least the primer oligonucleotide sequence must be conserved. DNA from a strain carrying *sup3-UCG* was digested with *Hin*dIII and ligated into the plasmid pTR262 (ref. 32) which allows positive selection for chimaeric plasmids. Plasmids harbouring *sup3-UCG* were identified by colony hybridization with a nick-translated probe carrying the *sup3-UGA* gene. Subsequently, a *Hin*dIII–*Bam*HI fragment of 950 bp carrying the *sup3-UCG* gene was transferred from the chimaeric plasmid to the phage M13mp8 (ref. 33). Single-stranded phage DNA served as template for nucleotide sequencing according to the dideoxy termination method[34] using as primer a synthetic tetradecamer; its nucleotide sequence is boxed. The primer (Applied Biosystems) was a gift of Dr D Söll. Detailed cloning and sequencing procedures will be described elsewhere.

UGA strain the new 4.6-kb fragment is still visible, while its two constituting subfragments of 3.4 and 1.2 kb length (as present in *sup12⁺* DNA) are missing (Fig. 4*b*). Surprisingly, no longer fragment appears in the corresponding *Hin*fI digests. When the sequences in the more distal parts of the introns of *sup12⁺* and *sup3-UGA* (Fig. 5) are examined, one discovers that there is the potential for the creation of a new *Hin*fI site in *sup12-UGA* by conversion. This explanation for the lack of a larger *Hin*fI fragment in *sup12-UGA* strains has to be tested by nucleotide sequencing.

The *sup3-UCG* convertant (presumed anticodon CGA) was derived from *sup3⁺* (anticodon TGA) as described above. But it has been shown that *sup3-UCG* convertants can also be obtained starting with *sup3-UGA* alleles (R. Aebi, C. Gysler & W. Heyer, personal communication). A new *Eco*RI site should be created in the *sup3-UCG* allele only if the convertant received its anticodon sequence from *sup12⁺* (anticodon CGA) together with at least part of the intron of *sup12⁺* (see Fig. 4*a*). In fact, the strains carrying the *sup3-UCG* allele yield a new shorter *Eco*RI fragment of 1.6 kb length (Fig. 4*b*). If a homologous *sup3* hybridization probe is used instead of the heterologous *sup12* probe an additional new fragment of 0.8 kb appears (data not shown). A band corresponding to the original *sup3* fragment of 2.4 kb (1.6 + 0.8 kb) is always visible, because it coincides with an *Eco*RI fragment from one of the other cross-hybridizing genes. Finally, the presence of the *Hin*fI site in the *sup3-UCG* allele (in contrast to *sup3-UGA*) is documented by the disappearance of the 2.2 kb *Hin*fI fragment in DNA from *sup3-UCG* strains (Fig. 4*b*). Instead, a new fragment of 0.7 kb length hybridizes to the probe, whereas the other subfragment of 1.5 kb length coincides with a fragment that stems from the *sup12* gene.

Our interpretation of these and further data not shown here is that multiple and nonadjacent base-pair changes occurred during the creation of the particular *sup12-UGA* and *sup3-UCG* alleles. This is best explained by intergenic conversion. To confirm this interpretation we are now cloning and sequencing the relevant genes. Very recent nucleotide sequence data concerning *sup3-UCG* actually show that in this intergenic convertant the whole intron sequence (differing at six nonadjacent positions from the *sup3⁺* intron sequence) and the sequence coding for the loop of the extra arm (differing at one position from *sup3⁺*) have been transferred from *sup12* to *sup3* (Fig. 5).

Discussion

From the evaluation of selfings of auxotrophic nonsense mutants carrying inactivated suppressors we conclude that recombinational information transfer occurs between the three unlinked serine tRNA genes *sup3*, *sup12* (both on chromosome I) and *sup9* (chromosome III) in *S. pombe*. This interpretation, in contrast to mutation, is discussed in detail in an earlier publication[19]. The crucial points are: (1) Much more prototrophs are obtained in selfings of inactivated suppressors (frequency range: 1.4×10^{-7} to 20×10^{-7}) than in controls that measure the mutational background (0.3×10^{-7}, Table 1). (2) In selfings of haploids the large majority of prototrophs (> 90%) are due to import of wild-type information into the marked tRNA gene. The gene thereby loses the inactivating secondary lesion and becomes suppressor active again. Only a minority of prototrophs (<10%, Table 1) are due to mutational events elsewhere in the genome. In selfings of diploid strains which permit to detect even recessive lethal events, we have now demonstrated in addition export of suppressor active anticodon information from the inactivated gene to other genes of the family. In this way a new opal suppressor has been obtained spontaneously that was never observed in controls (frequency: 1.7×10^{-7} in selfings of the inactivated allele *sup3-UGA,r36*). Restriction analysis and nucleotide sequencing has revealed multiple and nonadjacent base-pair changes in specific convertants.

The mechanisms responsible for information transfer between unlinked genes may be identical or related to the ones that operate in homologous recombination between alleles[14]. If this is true, it follows from the present results that blocks of conserved sequences as short as 200 bp embedded in unrelated sequences are sufficient for the initiation of hybrid-DNA formation, which with or without subsequent mismatch repair can result in gene conversion. This interaction may take place between short conserved sequences in the lateral DNA loops of different chromosomes.

Recently it has been shown *in vitro* that *recA* protein from *E. coli* is unable to extend hybrid-DNA formation through regions of DNA with an average of 1 mismatch per 10 bp[30]. Our own results show that eight mismatches within a stretch of 20 bp are no obstacle to exchanges between *sup3* and *sup12 in vivo*. It is true that suppressor anticodon export from *sup3-UGA,rX* to *sup12⁺* is only half as frequent as export to *sup9⁺* that carries an intron identical to *sup3* (Fig. 1). But there may be alternative explanations for this discrepancy, for example, effects due to the specific chromosomal location of the genes involved.

If events of intergenic conversion were frequently associated with crossing-over as in inter-allelic gene conversion, various chromosomal rearrangements would result and many such events would be lethal. In a first search no chromosomal rearrangements were identified among intergenic convertants (C. Gysler, personal communication). However, more has to be known about the orientation of the three tRNA genes with respect to their centromeres before the potential for the creation of viable rearrangements can be assessed.

More light may be shed on the mechanisms of intergenic conversion by studying other tRNA gene families. Experiments with suppressors derived from two leucine tRNA genes (not shown) indicate that they participate less frequently in intergenic conversion events. This may be due to shorter blocks of near-identical sequence in this gene family, since as a rule tRNA genes are not arranged in multimers in eukaryotes. This results in blocks of conserved sequence of not more than 100 bp.

Like inter-allelic recombination, intergenic conversion also seems to be influenced by specific marker effects. In the serine tRNA genes certain second-site mutations display vastly enhanced recombination frequencies in inter-allelic recombination[24]. The one such second-site mutant tested, *sup3-UGA,r10*, gives also the highest frequency of intergenic conversion (Table 1).

It has been shown experimentally that intergenic conversion occurs in various other systems[15–18] (see introduction). One additional example of *in vivo* formation of a 'hybrid' tRNA gene derived from two different tRNA genes has been discovered in the bacteriophage T4 (ref. 31 and W. McClain, personal communication). Intergenic conversion is likely to be the main mechanism responsible for concerted evolution of families of dispersed genes and also of linked genes[6,7,16,17]. It may propagate or eliminate specific mutations within a gene family and in this way contribute to conservative evolution and rapid sequence change. A recent review on the evolution of tRNA sequences includes a thorough discussion of the problems encountered in the construction of phylogenetic trees based on sequence comparisons[31]. Here we stress that the occurrence of intergenic conversion violates one of the fundamental assumptions used in the construction of phylogenetic trees, namely that the daughter copies will not exchange sequences after a gene has been duplicated.

The described selection of serine tRNA gene variants which are altered in intron sequences but remain functional at the tRNA level will prove useful for an understanding of tRNA biosynthesis (removal of intron sequences). From sequence data on inactive tRNA gene variants which carry secondary lesions introduced by either mutation or intergenic conversion one will also learn more about tRNA function.

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Identification of the class I genes of the mouse major histocompatibility complex by DNA-mediated gene transfer

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DNA-mediated gene transfer was used to identify cloned class I genes from the major histocompatibility complex of the BALB/c mouse. Three genes encoding the transplantation antigens H-2 K^d, D^d and L^d were identified as well as genes encoding the Qa-2,3 and two TL differentiation antigens. As many as 10 putative novel class I genes were detected by the association of their gene products with β_2-microglobulin. Alloantiserum prepared to one of the novel antigens was used to demonstrate the expression of the previously undetected antigen on spleen cells of various inbred, congeneic, and recombinant congeneic strains of mice.

THE major histocompatibility complex of the mouse encodes a family of cell-surface antigens known as the class I molecules. These gene products, which fall into two categories, are encoded by distinct regions of the major histocompatibility complex. The H-2 region encodes the transplantation antigens which were initially defined by allograft rejection[1,2]. These antigens are expressed on most somatic cells of the mouse and are involved in the T-cell recognition of cells altered by viral or neoplastic transformation (for a review see refs 3, 4). The second category of class I antigens are the haematopoietic differentiation antigens encoded by genes in the Tla region. The Tla region encodes three distinct types of differentiation antigens, Qa-1, Qa-2,3 and TL[5]. The TL antigens are expressed only on certain subpopulations of thymocytes and their

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Table 1 Summary of the coding assignments for the class I genomic clones

Clone	Reaction of transformants with antibodies specific for K^d	D^d	L^d	Qa-2,3	TL^c	Assignment
λ1.3	+					K^d
λ26.1	+					K^d
λ21.1	+					K^d
c17.1	+					K^d
c18.1		+				D^d
λ27.5			+			L^d
c59.2			+			L^d
c50.2				+		Qa-2,3
λ17.3					+	TL
c6.3					+	TL
λ24.8					+	TL
66.1					+	TL

Mouse L tk⁻ cells were co-transformed with ~1 μg of DNA from each of the λ and cosmid clones (see text) and the HSV tk gene in pBR322 (ptk5) as previously described[18]. For those cosmid clones containing more than one gene, the clone DNA was digested with an appropriate restriction enzyme to inactivate all but a single gene for transformation (see ref. 24). Uncloned tk^+ transformants selected in hypoxanthine/aminopterin/thymidine medium[38] were panel analysed by radioimmunoassay as previously described[18] using monoclonal hybridoma antibodies 34-1-2 to K^d (ref. 39), 34-2-12 to D^d (ref. 39), 30-5-7 to L^d (ref. 40), D3.262 to Qa-2,3 (ref. 41) and TLm(i) to TL[33]. Positives are indicated for those transformants which yielded at least 5,000 c.p.m. of iodinated (^{125}I) protein A bound (see Figs 2, 4) with a 10^{-2} or 10^{-3} dilution of the ascites fluid in duplicate transformation experiments. Background for the various antibodies and transformants ranged over 100 to 300 c.p.m., except for the IgM Qa antibodies which utilized a facilitating reagent (see Fig. 4). The active genes on cosmid clone 17.1, 18.1, 59.2, 50.2, 6.3 and 66.2 are the first gene on cluster 11, the first gene on cluster 13, the second gene on cluster 2, the second gene on cluster 6, the second gene on cluster 4, and the first gene on cluster 5, respectively (see Fig. 5).

neoplastic counterparts in certain T-cell leukaemias[6]. The Qa-1 antigen is expressed by thymocytes, peripheral T and B cells, as well as lymphoblasts[7]. The Qa-2,3 antigens are found on a subset of bone marrow-derived cells[8]. The biological functions of the TL or Qa antigens are not known.

Class I antigens have been characterized using specific alloantisera and monoclonal antibodies prepared by the appropriate cross-immunizations with cells from inbred, congeneic and recombinant strains of mice. Serological analysis has demonstrated that the transplantation antigens are extremely polymorphic and that different strains of mice have distinct combinations or haplotypes of class I alleles. Particular class I molecules are denoted by appropriate letters with a superscript small letter for the haplotype. For example, the serologically defined transplantation antigens of the BALB/c mouse which is of the $H\text{-}2^d$ haplotype are denoted K^d, D^d, L^d or R^d.

Class I molecules of both categories appear to share a common structure. The class I polypeptide is a 45,000-molecular weight integral membrane protein which is noncovalently associated with a 12,000-molecular weight polypeptide, β_2-microglobulin. Class I molecules consist of three external domains, each of ~90 amino acids, a transmembrane region of ~40 residues, and one cytoplasmic domain of 30 residues[9,10].

Class I cDNA clones have been characterized and used as probes to obtain genomic clones containing class I genes[11–17]. Two genes isolated from a BALB/c Crgl sperm DNA library constructed with the lambda bacteriophage (λ) vector have been extensively characterized. The class I gene from clone λ27.1 is a pseudogene which has been mapped to the Qa-2,3 subregion of the Tla region by restriction enzyme site polymorphisms[15]. DNA sequence analysis of gene 27.1 shows that this class I gene is divided into eight distinct exons encoding each of the individual domains of a class I molecule. Recently, DNA-mediated gene transfer and radioimmunoassays were used to demonstrate that a gene from clone λ27.5 encodes the $H\text{-}2L^d$ transplantation molecule[18]. The L^d molecules produced by mouse L cells transformed with λ27.5 DNA were shown to be virtually indistinguishable from the L^d molecules expressed on spleen cells from BALB/c mice by biochemical, immunological and functional criteria[18–21]. The DNA sequence of gene 27.5 is identical to that of the L^d antigen at all 77 positions that can be compared[22]. Other laboratories have also used gene transfer to identify the cloned class I genes from libraries constructed from tissues or tumour cell lines from other substrains[17] and strains[23] of mice.

In an attempt to assess the total number of class I genes contained in the BALB/c mouse, genomic clones containing class I genes have been isolated from a cosmid library constructed from BALB/c sperm DNA[24]. Fifty-four cosmid clones containing 36 unique class I genes have been assigned to 13 distinct groups or clusters encompassing 837 kilobases of DNA. These include most of the class I genes in the BALB/c mouse.

Here, we have used gene transfer and serological analyses to examine individual class I genomic clones from the λ and cosmid libraries. Cloned class I genes encoding the serologically defined H-2 K^d, D^d, L^d, Qa-2,3 and TL antigens have been identified. Evidence is also presented for as many as 10 additional novel class I genes encoding products not previously defined serologically.

Analysis of 96 λ and 36 cosmid class I clones by gene transfer

In an attempt to examine most class I genes from the BALB/c mouse, 96 genomic clones isolated from the λ library and 36 genomic clones previously isolated from the cosmid library (designated c) were analysed. DNA from each of these genomic clones, together with the herpes viral thymidine kinase gene, was used to co-transform thymidine kinase-negative (tk⁻) mouse L cells as previously described[18]. The class I $H\text{-}2^d$

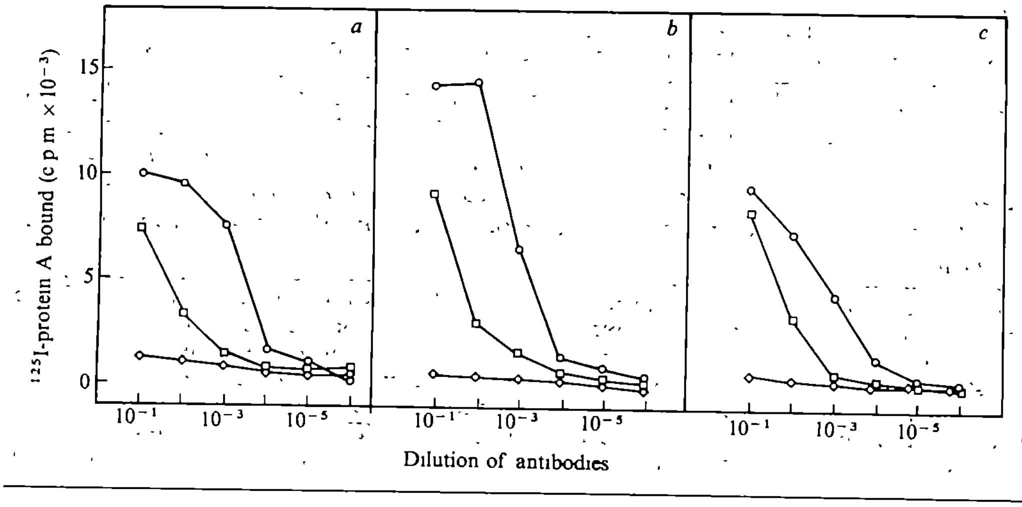

Fig. 1 Radioimmunoassay of λ1.3, λ21.1 and λ26.1 transformants. Transformants which reacted in the panel analysis (Table 1) with antibodies 34-1-2 to K^d molecules were tested by the cell-binding radioimmunoassay with: ○, 34-1-2 antibodies to K^d; □, 20-8-4 antibodies to K^b [42] which react with K^d molecules; and ◇, 34-2-12 antibodies to D^d. Transformants derived from transfection with DNA from clones λ26.1 (*a*), λ21.1 (*b*) and λ1.3 (*c*). Spleen and Ltk^+ controls are shown in Fig. 4.

products of the transformed L cells were detected by radioimmunoassay and analysed by two-dimensional gel electrophoresis. Since mouse L cells are fibroblasts derived from C3H mice of the *H–2*^{k} haplotype, the endogenous K^k and D^k transplantation antigens can readily be distinguished from any of the BALB/c class I molecules of the *H–2*^{d} haplotype by appropriate monoclonal antibodies. The mouse L-cell fibroblasts do not express the differentiation antigens Qa-1, Qa-2,3 or TL. These results are summarized in Table 1. In almost all cases, the binding of specific antibodies to the transformants was comparable to that of the binding of the antibodies to the corresponding antigens and not cross-reacting antigens (see Fig. 4).

Identification of genes encoding the H–2 K, D and L transplantation antigens

The L-cell transformants derived from transfections with the λ1.3, λ26.1 and λ21.1 clones express K^d molecules which are virtually indistinguishable in their patterns of serological reactivity with two different monoclonal antibodies to the K^d molecule (Fig. 1), both of which react as strongly with these transformants as they do with spleen cells (see Fig. 4). No reactivity is observed with these four transformants using monoclonal antibodies specific for D^d or L^d antigens (see Fig. 1 legend). Antibodies specific for the K^d molecule do not react with mouse L cells transformed only with the thymidine kinase gene (see Fig. 4). These are designated tk^+ transformants. To determine whether these three class I genes encode distinct gene products, the antigens were isolated by immunoprecipitation and analysed by two-dimensional gel electrophoresis (Fig. 2). As the class I antigens of different haplotypes each have distinct constellations of spots[25], we believe this approach is very discriminating. The products of class I genes 1.3, 26.1 and 21.1 appear extremely similar to one another and to the K^d molecules isolated from normal BALB/c spleen lymphocytes. However, minor electrophoretic differences were observed in the faint, lower molecular weight species (Fig. 2*a* and 2*e*).

As the electrophoretic patterns between products obtained from duplicate transformations appear identical, the variation in the lower molecular weight products may reflect differences in post-translational modifications of these molecules or differential alternative splicing, as has been previously suggested for class I genes[15]. Restriction map analyses of clones λ1.3, λ26.1 and λ21.1 (Fig. 3), however, suggest that the K^d genes they contain are identical to one another and also to the first gene on cluster 11 or clone c17.1 (see Fig. 5) which contains a K^d gene by transformation (Table 1, Fig. 4*a*). Thus, all of these clones appear to encode very similar or identical genes on an independently derived series of what we believe are overlapping clones, although we cannot exclude the possibility that certain of these genes may actually be distinct and yet appear identical by the limited restriction mapping we have carried out to date. These genes are currently being sequenced in order to resolve this issue.

Cosmid clone 18.1 contains an H–2D^d gene as demonstrated by the radioimmunoassay of the c18.1 transformants with monoclonal antibodies to theD^d antigen (Table 1, Fig. 4*b*). The 18.1 transformants reacted with antibodies specific for D^d products and not reagents which detect K^d, L^d, TL or Qa antigens. The D^d molecules expressed by the transformants are currently being compared with the molecules isolated from spleen cells by two-dimensional gels and tryptic peptide map analysis to confirm this identification. No lambda clones containing an H–2 D^d gene were identified (see below).

An H–2L^d gene has been identified on cosmid clone 59.2 by transformation (Table 1) and comparison of restriction maps between the c59.2 and λ27.5 genes[24]. The L^d molecules expressed on the corresponding transformants appear indistinguishable in radioimmunoassays from the products of the previously identified gene contained in clone λ27.5 (Fig. 4*c*).

The analyses based on 132 independent transformations resulted in the identification of at least one H–2K^d, a single H–2D^d and one H–2L^d gene. Serological evidence suggests that in some mice there may be multiple K and D gene products[26–31], and additional transplantation antigens such as R^d (ref. 32).

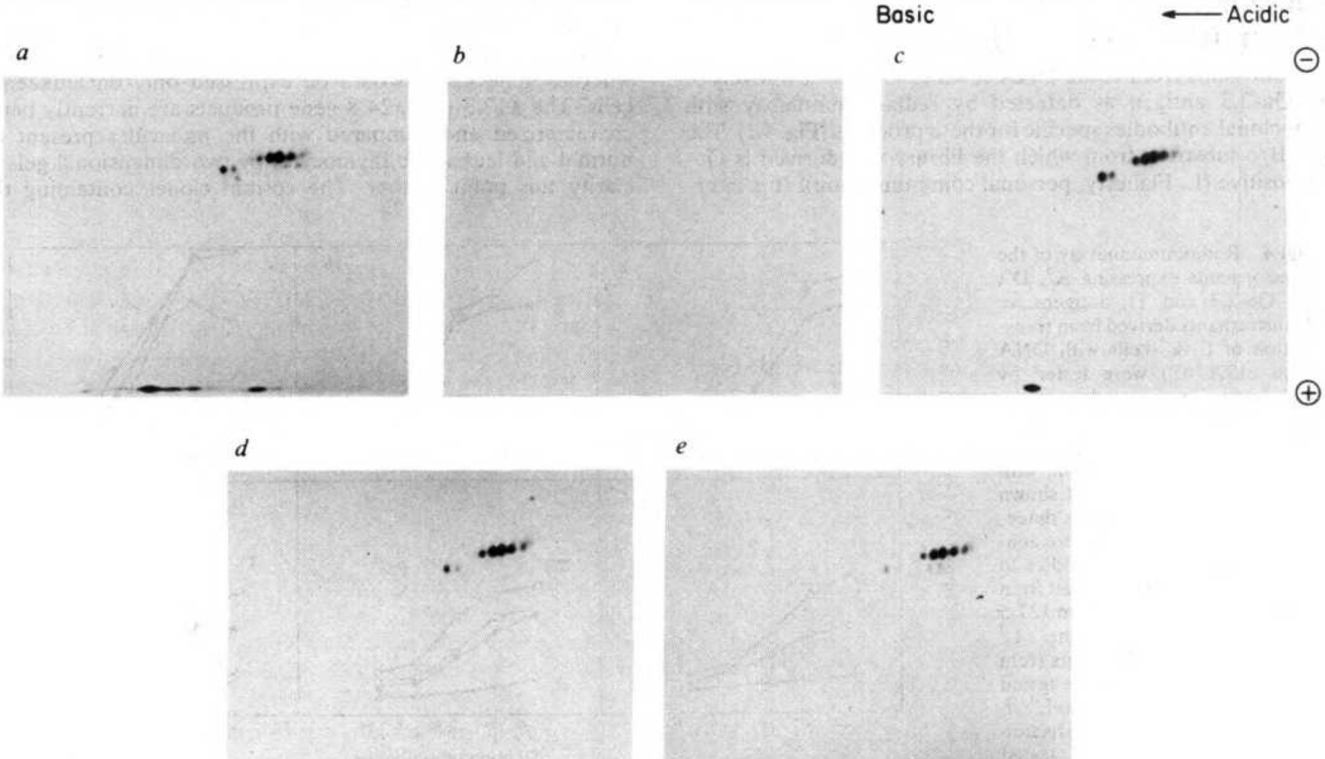

Fig. 2 Fluorographs of two-dimensional gels of H–2K^d molecules immunoprecipitated from cell lysates of BALB/cJ spleen lymphocytes (*a*), tk^+ cells transformed only with the tk gene (*b*), λ1.3 (*c*), λ26.1 (*d*) and λ21.1 (*e*) transformants. Cells were biosynthetically radiolabelled, immunoprecipitated with 20-8-4 monoclonal antibodies (supernatant fluids), and analysed by two-dimensional gel electrophoresis as previously described[18]. The gels were prepared for fluorography, dried and exposed for ~7 days.

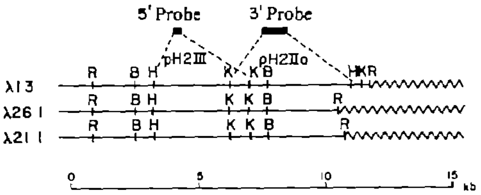

Fig. 3 Restriction maps of the lambda clones containing the K^d genes. DNA from each of the λ clones which yielded K^d-positive transformants was restriction-mapped with the indicated enzymes by single and double digestion as previously described[24]. The 5' probe pH-2III and the 3' probe pH-2IIa[11] were used to detect fragments containing coding sequences homologous to the class I genes (dashed lines). The pH-2III probe contains the sequences encoding amino acids 63–160 of a transplantation antigen and pH-2IIa amino acids 167–352. The coding sequences were orientated using sequencing subclones as previously described[22]. The solid line indicates the insert DNA, and the λ vector DNA is designated by the wavy lines. R, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; K, *Kpn*I. The 3' *Eco*RI sites mark the linkers used in constructing the library.

The failure to find multiple class I K^d and D^d genes may be explained in several ways. First, the serological multiplicity of K or D antigens may arise from post-translational modifications or alternative patterns of RNA splicing. Second, different inbred strains may have different numbers of K and D genes. Third, multiple class I genes might appear identical by restriction mapping and still differ in their DNA sequences. In this case, multiple distinct genes might be confused with a single gene present on overlapping clones. Finally, these genes may not have been detected for various technical reasons, for example, failure to clone the gene or express the gene at levels detectable in the radioimmunoassay. For example, the radioimmunoassay is less effective with heterogeneous alloantisera and specific monoclonal reagents for the R^d or the Qa-1 gene products are lacking.

Fibroblast expression of gene encoding the mouse haematopoietic differentiation antigen Qa-2,3

Transformants from c50.2 DNA seem to express low levels of the Qa-2,3 antigen as detected by radioimmunoassay with monoclonal antibodies specific for these products (Fig. 4*d*). The BALB/c substrain from which the library was derived is Qa-2,3-positive (L. Flaherty, personal communication). It is interesting that *Qa-2,3*, a gene normally expressed only in haematopoietic cells, appears to be expressed in transformed fibroblasts. The fact that the Qa-2,3 differentiation antigen is expressed at low levels on haematopoietic cells[8] may explain why limited numbers of Qa-2,3 molecules are expressed in the transformed mouse L cells. It is difficult to ascertain the basis for the low level of expression of this antigen by the transformants. Several possibilities are now being investigated. For example, only rare transformants may have incorporated sufficient copies of the gene to override differentiation signals. In addition, glycosylation of the polypeptide in fibroblasts might, in this particular instance, alter the antigenicity of the molecule causing a less avid reaction with the antibodies. The fact that the c50.2 transformants exhibit elevated levels of β_2-microglobulin (see below) does suggest that significant amounts of the antigen may be expressed in an altered form associated with β_2-microglobulin. Accordingly, it has been difficult to obtain sufficient Qa-2,3 antigen from spleen cells or transformed L cells for two-dimensional gel analysis. The identification of a Qa-2,3 gene should readily permit its characterization at the DNA level.

Two different class I genes encode two distinct TL T-cell differentiation antigens

The TL antigens are expressed only on thymocytes at a certain stage of differentiation. In some inbred strains of mice, distinct TL molecules are expressed on certain leukaemic cells of T-cell origin[6]. Two monoclonal antibodies can distinguish these TL antigens. Antibodies denoted TLm(i) recognize TL determinants present on both normal and leukaemic cells, whereas antibodies designated TLm(iii) react only with the TL determinants on normal thymocytes[33]. Figure 4*e*, *f* shows that transformants from λ clone 17.3 reacted with both monoclonal antibodies at levels comparable to those observed with BALB/c thymocytes (Fig. 4*e*, *f*), whereas transformants from λ clone 24.8 reacted only with monoclonal antibodies TLm(i). These observations suggest that there are at least two distinct genes, λ17.3 and λ24.8, which encode two TL antigens. From the serological analyses described above, gene λ17.3 may be expressed on normal BALB/c thymocytes and leukaemic cells whereas gene λ24.8 would be expressed only on leukaemic cells. The λ17.3 and λ24.8 gene products are currently being characterized and compared with the molecules present on normal and leukaemic thymocytes by two-dimensional gels to clarify this point further. The cosmid clones containing the

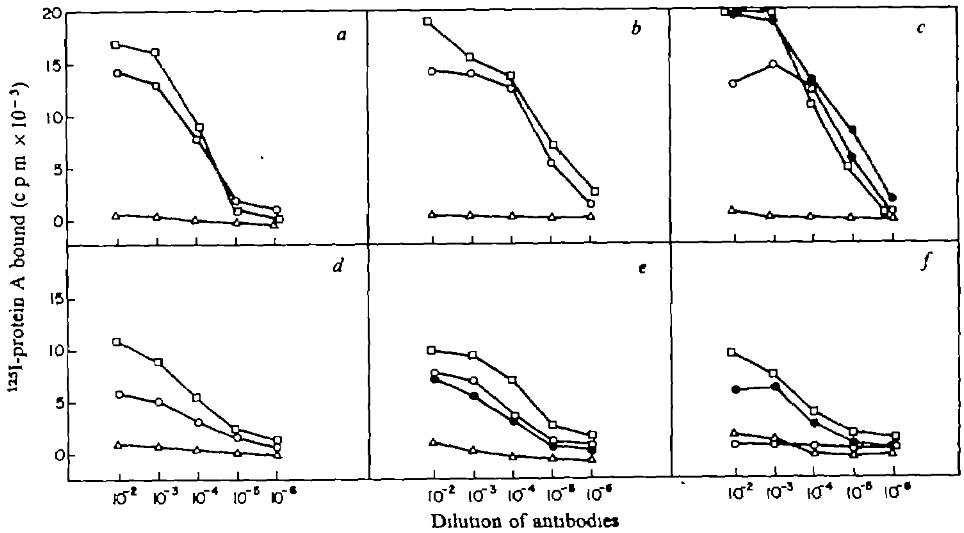

Fig. 4 Radioimmunoassay of the transformants expressing K^d, D^d, L^d, Qa-2,3 and TL antigens. *a*, Transformants derived from transfection of L tk$^-$ cells with DNA from c17.1 (○) were tested by radioimmunoassay for the expression of K^d molecules using 34-1-2 antibodies to K^d. *b*, Transformants from transfection with DNA from c18.1 (○) and shown to express D^d molecules as detected in the panel analysis were analysed using 34-2-12 antibodies to D^d. *c*, Transformants derived from transfection with DNA from λ27.5 (○), which contains the L^d gene[18,22], and transformants from DNA from c59.2 (●) were tested against 30-5-7 antibodies to L^d. *d*, Transformants from transfection with c50.2 DNA (○) were tested in a cell-binding radioimmunoassay using D3.262 monoclonal hybridoma IgM antibodies to Qa-2. Rabbit antiserum to mouse IgM (Bionetics) was used as a second step reagent before incubation of the cells with ^{125}I-protein A. *e*, Transformants derived from transfection with DNA from λ17.3 were tested against antibodies to TL (●) TLm(i) and (○) TLm(iii) (see text). *f*, Transformants from transfection with λ24.8 DNA were tested against the same antibodies to TL indicated in *e*. BALB/c spleen cells, panels *a*–*d* (□) or thymocytes, panels *e*–*f* (□) and Ltk$^+$ cells, panels *a*–*f* (△) were run as controls. Thymocytes and Ltk$^+$ cells in panel *e* were tested against TLm(i), panel *f* against TLm(iii).

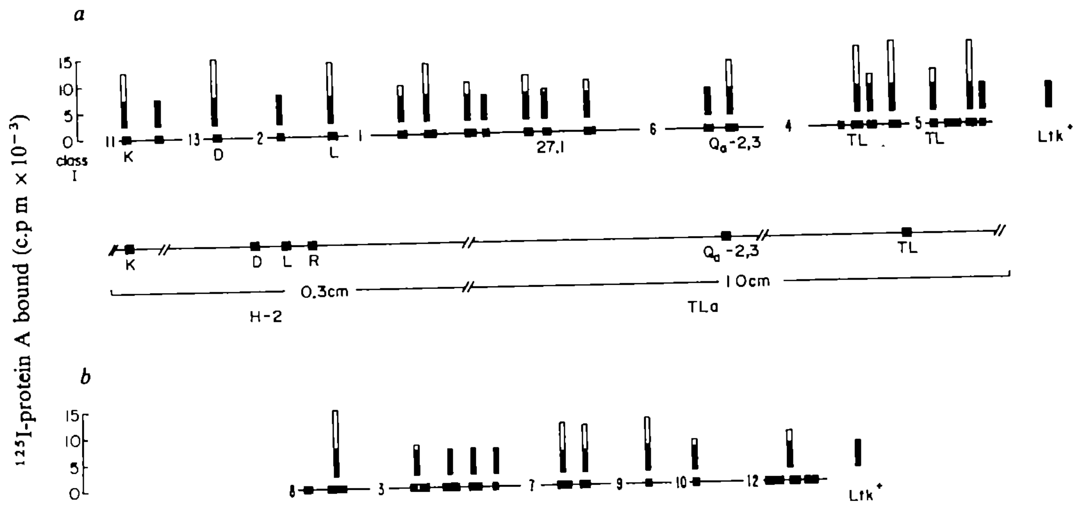

Fig. 5 Radioimmunoassay of cosmid transformants for β_2-microglobulin. The mixed population of tk$^+$ transformants derived from transfection with a single gene from each of the various cosmid clones, obtained by restriction digestion, was tested for the expression of β_2-microglobulin in a cell-binding radioimmunoassay using rabbit antiserum to rat β_2-microglobulin. Each transformant was assayed in duplicate against a 10^{-2} dilution of the antiserum. The numbers shown at the left indicate the c.p.m. of ^{125}I-protein A bound per 50,000 cells. Less than 10% variation in the number of c.p.m. was observed between duplicate transformations or assays. The dark portion of each bar represents the basal level of L-cell β_2-microglobulin measured for tk$^+$ transformants which is the average of duplicate samples tested (right). The open portion of each bar represents the total number of c.p.m. of ^{125}I-protein A bound for each transformant which exceeds the level on tk$^+$ cells. Cosmid clusters are represented schematically with the genes indicated as the enclosed boxes. Each cluster is comprised of a group of noncontiguous overlapping clones[24]. The cosmid cluster number is indicated to the left of the horizontal black line and the genes encoding the serologically defined products are indicated below the appropriate boxes. *a*, β_2-microglobulin on the surface of transformants derived from transfection with DNA from cosmid clones linked with mapped genes or genes encoding serologically defined antigens; *b*, undefined clusters only.

corresponding TL genes have been identified by transformation (Table 1, Fig. 5). The 17.3 TL gene appears to be the first gene on cosmid cluster 5 or clone 6.3, and the 24.8 gene the second gene on cosmid cluster 4 or clone 66.1. Since each TL gene maps to a separate cosmid cluster, they must be distinct genes. These results illustrate two important points regarding gene transfer into mouse L cells. First, as was noted for the Qa-2,3 gene, the TL genes which are developmentally regulated and expressed only on thymocytes can be expressed in fibroblasts as a result of transformation. Second, genes not expressed in the mouse except on leukaemias, such as gene λ24.8, can be expressed by transformed fibroblasts. It seems unlikely that the activation of endogenous L-cell genes encoding similar TL molecules would account for these results as restriction digestion of λ17.3 or λ24.8 DNA destroys the ability of these genes to transform L cells to the TL-positive phenotype.

Ten class I genes encode serologically undefined gene products

Of the 36 distinct class I clones, six contain genes encoding serologically defined gene products (Fig. 5*a*). The remaining class I genes are either pseudogenes or encode as yet unidentified cell-surface products. For example, they could encode nonpolymorphic molecules expressed on the surface of most cells which therefore would not have been detected by classical serological approaches. Alternatively, they might be differentiation antigens expressed at defined stages on tissues not yet tested for their ability to generate alloantisera. Cytoplasmic or secreted forms of class I molecules might represent another category of uncharacterized products.

We have used a simple but effective approach to identify class I gene products for which there are no specific serological reagents. All class I molecules studied associate with β_2-microglobulin before expression on the cell surface[34,35]. Therefore, the expression of foreign class I molecules on the surface of the mouse L cell should result in the elevation of the amount of β_2-microglobulin expressed on the cell surface. To test this hypothesis, a radioimmunoassay for cell-surface β_2-microglobulin was developed and used to quantitate the amount of β_2-microglobulin on several transformants expressing foreign serologically defined class I molecules as well as several which failed to react with any of the class I-specific antibodies. The tk$^+$ transformants express a basal level of β_2-microglobulin as do the mixed population of tk$^+$ transformants derived from transfection with the L^d gene cleaved into small coding fragments with *Bam*HI enzyme. In contrast, mouse L cells transformed with the L^d, D^d, K^d and TL genes all expressed levels of cell-surface β_2-microglobulin well above the background level as measured by radioimmunoassay (Fig. 5*a*). This increase is reproducible in that the measured levels of β_2-microglobulin obtained for duplicate assays or transformations vary by less than 10%. In addition, the elevation in β_2-microglobulin expression appears to be a stable phenotype, observed after 50 passages of some of the cell lines in culture. If elevated β_2-microglobulin expression results from the expression of additional class I molecules by the transformants, this phenotype would be stably expressed because it has been demonstrated

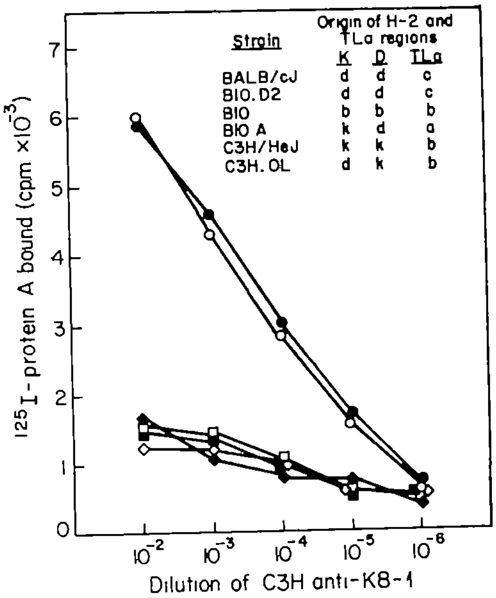

Fig. 6 Radioimmunoassay of spleen cells with C3H antibodies to K8-1 transformants. Four C3H mice were immunized by injecting 5×10^7 UV-irradiated K8-1 cells intraperitoneally every 2 weeks for 8 weeks. Mice were bled 7 days after the last injection and the antiserum used in the cell-binding radioimmunoassay against spleen cells as previously described[18]. Reaction of spleen cells from: ●, BALB/cJ; ○, B10.D2; ■, C3H/HeJ; □, C3H.OL; ◆, B10; ◇, B10.A. The origins of the H–2 and Tla regions for each of the mice are indicated in the panel.

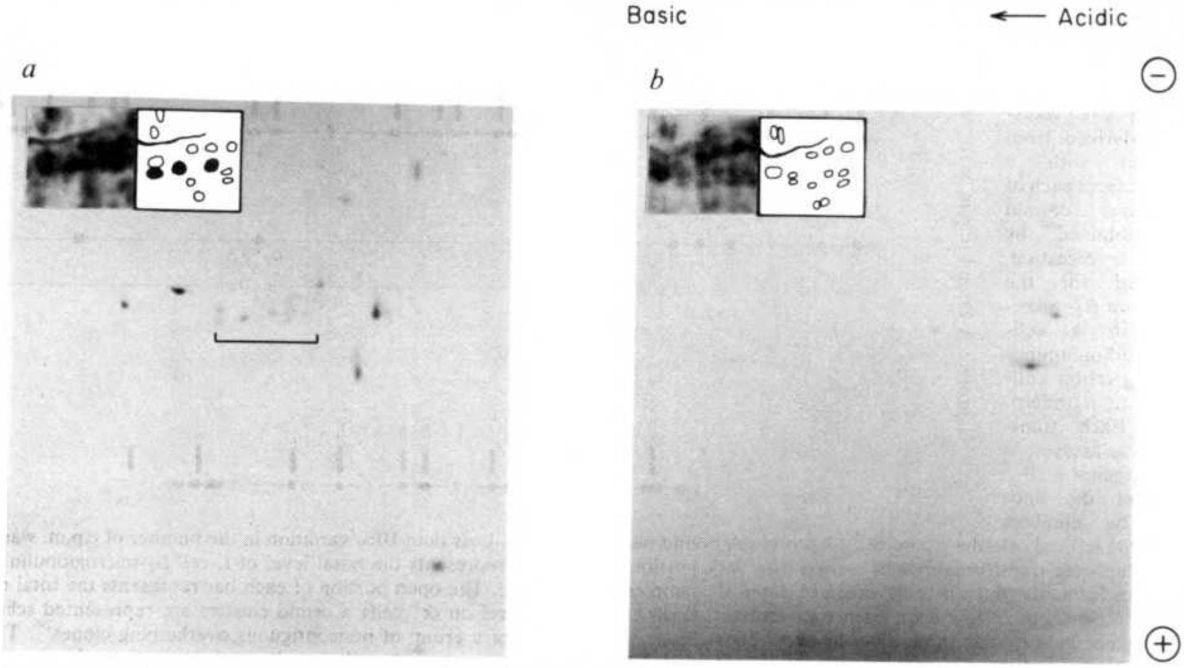

Fig. 7 Fluorographs of two-dimensional gels of the products of the c2.1 gene immunoprecipitated from K8-1 cells (*a*) and tk^+ transformants (*b*) using C3H antibodies to K8-1 transformants. The antibodies prepared in C3H mice against c2.1 transformants (K8-1 cells) were used to immunoprecipitate radiolabelled antigens for two-dimensional gel electrophoresis as previously described[18]. The insets contain longer exposures and diagrammatic representations of the 45,000-molecular weight region of each gel. Black spots represent the specific immunoprecipitated molecules from 8-1 cells.

previously that the expression of the transferred genes is a stable phenomenon[18].

The ability to measure an elevation in β_2-microglobulin expression by the pooled transformants is related to the fact that in the conditions used for transformation, nearly 100% of the co-transformants express significant and comparable levels of the foreign class I molecules[36]. It is important to stress that the twofold increase in the magnitude of the radioimmunoassay signal does not necessarily correspond with a twofold increase in the amount of β_2-microglobulin expressed (see ref. 36). These results suggest that the L cells possess the capacity to express additional class I molecules. It has recently been shown that the expression of foreign class I molecules does not occur at the expense of the endogenous $H\text{-}2^k$ products of the transformants[36]. Furthermore, we have observed significant levels of expression of as many as three different products by the triple K^d, D^d and L^d cloned transformant line, which demonstrates even greater than a twofold increase in the β_2-microglobulin radioimmunoassay signal.

At least 10 of the transformants which exhibited increased levels of β_2-microglobulin expression failed to react with any of the known serological reagents to class I molecules (Fig. 5*a*,*b*), suggesting that these cells produced novel products associated with β_2-microglobulin. Unfortunately, the heterologous antibodies to β_2-microglobulin proved relatively ineffective in precipitating class I molecules for two-dimensional gel analysis. Thus, we decided to prepare antisera directly against several of these gene products in order to demonstrate formally the presence of novel class I genes. Mouse L cells transformed with the $H\text{-}2L^d$ gene have been used to immunize C3H mice, the strain from which the L cell was derived, to generate alloantisera specific for the L^d molecule[20]. Similarly, C3H mice were immunized with L-cell transformants (K8-1) obtained by gene transfer with cosmid clone 2.1, the first gene on cosmid cluster 1 (see Fig. 5*a*). By radioimmunoassay, the antibodies specific for K8-1 cells reacted weakly with BALB/c spleen cells, whereas significantly lower reactivity was observed against BALB/c liver or thymus cells, and C3H spleen, liver or thymus cells (Fig. 6). Accordingly, these antibodies constitute an alloantiserum that was then used to screen spleen cells from a panel of different congeneic and recombinant congeneic strains of mice so that the location of gene c2.1 in the major histocompatibility complex could be assigned by preliminary mapping. These serological analyses suggest that gene c2.1 maps to the right of the complement region. More extensive mapping is required to pinpoint the location of this gene by serological analysis. Such analysis may be complicated if only certain strains of mice express the 2.1 gene products in a manner analogous to that of Qa or TL antigens. However, the data are consistent with the mapping of this gene by restriction enzyme polymorphisms, in that class I gene c2.1 is the first gene in the cosmid cluster containing gene 27.1, which had previously been mapped to the Tla region[15] (Fig. 5*a*).

The antiserum to the K8-1 cells immunoprecipitated molecules from K8-1 cells but not from tk^+ transformants or BALB/c spleen cells (Fig. 7). The products of the c2.1 gene appear to be ~45,000 molecular weight and to consist of a number of spots of the appropriate *p*I in a sequential array somewhat characteristic of class I molecules analysed on two-dimensional gels (see Fig. 2). β_2-Microglobulin could not be readily detected in 8-1 immunoprecipitates. This may be due to a low affinity between β_2-microglobulin and the 2.1 gene products, as has been described for certain $H\text{-}2^d$ molecules[37]. The origin of the additional products precipitated is unknown. The fact that these products cannot be isolated from spleen cells may be due to the fact that this antigen is expressed at low levels on lymphocytes. However, the ability to generate antibodies to the putative novel gene products expressed by the K8-1 transformants supports the existence of novel genes. Moreover, these genetic, serological and chemical data suggest that gene c2.1 encodes a class I molecule which may, because of its location within the major histocompatibility complex, be a differentiation antigen and not a ubiquitously expressed transplantation antigen.

Mapping of six serologically defined genes

The serologically defined class I genes have been localized within the major histocompatibility complex through the serological analysis of the highly polymorphic congeneic mouse strains. Thus the identification of these class I genes by DNA-mediated gene transfer allows us to map precisely the corresponding cosmid clusters into the major histocompatibility complex. It is interesting that only five class I genes shown in Fig. 5 map to the H–2 region (clusters 2, 11 and 13). Three class I genes encode defined transplantation antigens whereas the remaining two apparently do not encode detectable class I gene products. In contrast, 17 class I genes are contained in cosmid clusters which map to the Tla region. Four class I genes encode serologically defined differentiation antigens and eight encode novel gene products. One of the eight novel genes (c2.1) may encode a differentiation antigen based on the pattern of tissue distribution determined with the alloantibodies. Therefore, the Tla region contains far more class I genes than the H–2 region.

Cosmid clusters 3, 7, 8, 9, 10 and 12 do not express serologically defined gene products and, accordingly, cannot be mapped within the major histocompatibility complex (Fig. 5*b*). These six cosmid clusters contain 15 class I genes, six of which appear to express novel gene products. These clusters are now being mapped through the analysis of restriction enzyme site polymorphisms.

Conclusion

We have identified at least one H–2 K^d, D^d, L^d, possibly a Qa-2,3, and at least two TL genes from the BALB/c mouse. These genes have been mapped into 6 cosmid gene clusters containing 14 class I genes (Fig. 6), of which 9 lie in the Tla region and 5 in the H–2 region. As many as 10 putative novel class I gene products, detected on the basis of their association with β_2-microglobulin, may be encoded by genes dispersed between the mapped and unmapped cosmid gene clusters. In one case, antibodies to a novel class I antigen apparently detect the corresponding antigen expressed at low levels on BALB/c spleen cells.

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LETTERS TO NATURE

Measurements on a shock wave generated by a solar flare

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Shock waves generated by intense solar flares may be driven by a large amount of ejected mass, about 5×10^{16} g, and the total energy involved may be of the order of 10^{32} erg. The shocks may have initial velocities of the order of 2,000 km s^{-1} and, in their exodus through the corona, may be accompanied by fast-moving optical transients, the emission of highly characteristic radio signatures and the acceleration of particles to quasi-relativistic velocities[1]. Here we review data on a high-velocity shock generated by a flare on 18 August 1979, 1400 UT, comment on some previously deduced velocities for the shock[2], and then present a model, based on current computer programs to account for the overall characteristics of the shock as it propagated through the corona and the interplanetary plasma.

The solar flare that occurred on 18 August 1979 at 1400 UT was one of the more energetic flares of the current solar cycle. Optically, the flare was not well observed because it occurred just behind the east limb at heliographic latitude N08°. Nevertheless, it gave rise to significant X-ray and radio emission, as well as to a high-velocity shock. The shock was tracked by its radio emission of spectral type II, first by radio spectral equipment (25–580 MHz) at the Harvard Radio Astronomy Station, Fort Davis, Texas, and then by low-frequency equipment (0.03–2 MHz) on the ISEE 3 satellite[3]. It impinged on the Earth's magnetosphere some 40 h later, giving rise to the sudden commencement of a magnetic storm. Data on the outward-travelling shock were also acquired at a point $13.1R_0$ from the Sun by a technique described by Woo and Armstrong[2]. Their velocity estimates were obtained by examination of the effects of the shock and its driver-piston on signals transmitted at 2.3 and 8.4 GHz from the Voyager 1 spacecraft and received on Earth. At the time of the flare, the line of sight from Voyager 1 to Earth passed within 13 R_0 of the Sun and, when the shock passed this point, abrupt changes began in the radio signals received from the spacecraft, as may be seen in Fig. 1.

In applying the Rankine–Hugoniot relations to determine the shock velocity at 13 R_0, Woo and Armstrong suggested using a pre-shock velocity of 200 km s^{-1}, with which we agree,

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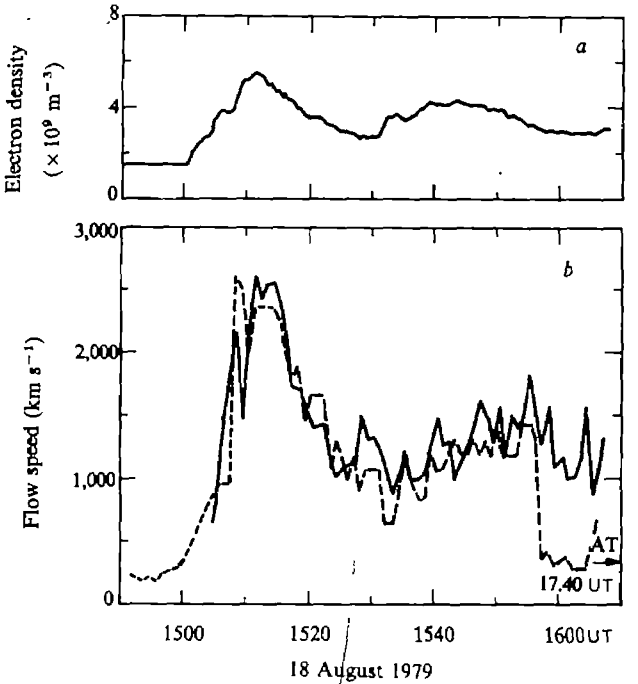

Fig. 1 Plasma data at 13.1 $R_\odot$ ($R_\odot$ = radius of the Sun) derived from changes in radio transmissions from Voyager 1, at the time of passage of the flare-generated shock. *a*, Changes in electron density; *b*, changes in flow speed (after ref. 2). Dashed line, log amplitude scintillations; solid line, spectral broadening.

and a post-shock velocity of 2,600 km s^{-1}, which, based on the data of Fig. 1, we believe to be too high. We suggest that the flow velocity of 2,600 km s^{-1} measured at 1510 UT by Woo and Armstrong refers to the plasma velocity substantially behind the shock, whereas the proper application of the Rankine–Hugoniot relations requires use of a velocity immediately behind the shock. We suggest the shock probably passed the 13 R_0 point at 1500 UT, that its passage probably took <10 s, and that everything seen thereafter in Fig. 1 refers to the region of intense turbulence behind the shock. The 10-s period also represents an upper limit and is derived on the assumption that the shock thickness is, at most, of the order of 1–10 ion gyroradii[4,5]. In view of our ignorance of magnetic field strength and direction in the shock, we use a roughly equivalent estimate of 10–100 ion inertial lengths[4,5]. Using the pre-shock electron density of 1.5×10^9 electrons m^{-3} given by Woo and Armstrong, and assuming that the electron density equals the ion density, we find the ion inertial length ($c/\omega_{\rm pi}$, where c is the velocity of light and $\omega_{\rm pi}$ the ion plasma frequency) to be 6 km and hence deduce the shock thickness to be of the order of 60–600 km. For a shock travelling at about 2,000 km s^{-1} the front would thus pass the line of sight in ~1 s—literally in a flash. We therefore choose the period 1502–1504 UT in Fig. 1, to determine the post-shock velocity, which would thus be of the order of 1,000–1,500 km s^{-1}. Assuming conservation of mass through the shock and the pre-shock electron density and velocity given by Woo and Armstrong, we then determine the shock velocity as follows. Assume that the pre-shock velocity $v_1 = 200$ km s^{-1}, the post-shock velocity $v_2 = 1{,}250$ km s^{-1}, the pre-shock electron density $n_1 = 1.5\times10^9$ electrons m^{-3}, and the post-shock electron density $n_2 = 3.0\times10^9$ electrons m^{-3}. The shock velocity then equals $(v_2n_2 - v_1n_1)/(n_2 - n_1) = 2{,}300$ km s^{-1} approximately, rather than the value of 3,500 km s^{-1} derived by Woo and Armstrong.

In a second estimate of the shock velocity, based on its transit time from the flare region to 13 R_0, Woo and Armstrong noted that "shock arrival occurs at approximately 1501 UT and based on the transit time from the Sun to 13.1 R_0 we obtain a shock speed of 3,509 km s^{-1}", but they made no specific reference to the starting time of the shock in the vicinity of the solar flare. There are, however, four separate lines of evidence that indicate that the explosive phase of the flare, which we take to be the starting time of the shock, occurred at approximately 1402 UT. A transit time of 60 min for a shock to reach 13 R_0 then translates into an average velocity of 2,350 km s^{-1}. The evidence relating to the explosive phase of the flare, shown in Fig. 2, is as follows: (1) an abrupt increase in radio centimetric emission began at approximately 1402 UT and this was followed by a burst of 45 min duration and of considerable energy. (2) Hard X-ray emission showed a sudden increase over the period 1359–1401 UT. (3) A metric radio type II burst was recorded over the period 1412–1423 UT and statistical surveys repeatedly show that type II bursts in the metre band are first seen about 6–10 min after the explosive phase of a flare[6] (this delay corresponds to the time taken by a shock to travel from the region of energy release in the low corona to the heights at which metric type II radio bursts are generated), which again suggests a start time for the shock of about 1402 UT. (4) Type III radio bursts, probably associated with the explosive phase of the flare, were recorded at 1402–1404 UT. (Figure 2 also shows a small radio burst in the centimetre band and an accompanying X-ray burst at 1345 UT. We believe, as do other investigators (S. Kane personal communication), that this burst was a precursor to the main event. If it did, however, signify the start time of the shock, the transit time of the shock to 13 R_0 would be 15 min longer and the deduced average shock velocity would be even less than 2,350 km s^{-1}.)

A shock velocity of 2,300 km s^{-1}, rather than the velocity of 3,500 km s^{-1} derived by Woo and Armstrong, agrees somewhat better, we feel, with existing models for propagation of flare-generated shocks from the Sun to the Earth. For this particular flare, the propagation time of the shock between the Sun and the Earth was 40 h. (The shock reached the ISEE 3 satellite, located at the lagrangian point between the Sun and the Earth,

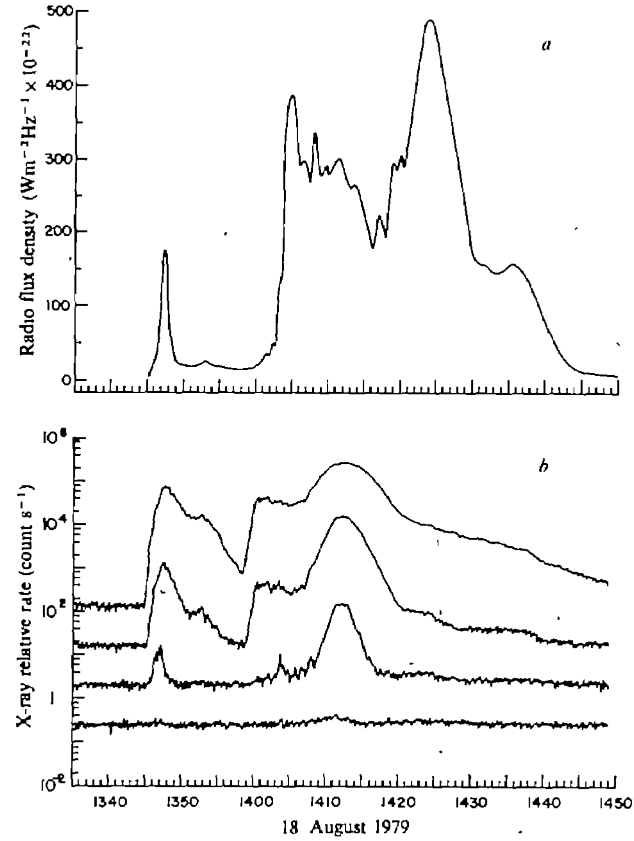

Fig. 2 Radio and X-ray emission from E-limb solar flare of 18 August 1979, 1400 UT. *a*, Radio burst at 2,800 MHz. *b* X-ray bursts recorded by ISEE 3 satellite; reading from top to bottom, the X-ray curves cover the energy ranges 26–43, 43–78, 78–154 and 154–398 keV, respectively.

on 20 August 1979 at 0550 UT[2] and the Earth at 0625 UT.) Bearing in mind that the flare occurred at heliographic longitude ~E 90, we suggest that, to reach the Earth, the shock must have had a wide angular extent and very considerable energy. We suggest that it had an initial velocity of the order of 2,300 km s^{-1} and that, while ascending through the corona it was accelerating electrons to quasi-relativistic energies and generating X rays (identifiable in Fig. 2 by the marked increase in X rays over the period 1407–1420 UT, at the time of the observed type II radio burst). The shock was then driven at a constant velocity of ~2,300 km s^{-1} to a distance of about 75 R_0 (S. Kane, personal communication), after which it propagated through the solar wind to the Earth as a blast wave, reaching the Earth ~40 h after the flare with a velocity of 750 km s^{-1}. The average deceleration between 75 and 215 R_0 was thus 0.012 km s^{-2}, which is consistent with previously estimated values for the deceleration of shocks between the Sun and the Earth[7,8]. We also note that the wide helio-longitudinal extent of the shock probably represents the rule, rather than the exception, for flare-generated shocks.

The X-ray data in Fig. 2 are reproduced by kind permission of Dr Sharad Kane, Space Sciences Laboratory, University of California, Berkeley. The centimetric radio data in Fig. 2 were provided by courtesy of Dr M. B. Bell, Astrophysics Branch, National Research Council, Ottawa. The work of Alan Maxwell described in this paper was carried out under NASA grant NSG-7648 and NSF grant ATM 80-07024.

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Coronagraphic observations of two new sungrazing comets

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We recently reported the discovery[1] of a comet (Howard-Koomen-Michels: 1979 XI) that apparently collided with the Sun on 30 August 1979. We now report observations of two additional sungrazers that encountered the Sun on 27 January 1981 and 20 July 1981, respectively. Like comet 1979 XI, these two new comets seem to have been members of the Kreutz group of sungrazers[2–4], and like 1979 XI the new comets did not reappear after their encounters with the Sun. The discovery of three previously unreported comets during the initial 2.3 yr of our satellite coronal observations leads us to suspect that sungrazers are much more common than one might suppose from the list of only nine known sungrazers observed during the years 1668–1970[5,6].

Our observations were obtained with the Naval Research Laboratory's Earth-orbiting SOLWIND coronagraph. This instrument has been operating routinely on the US Air Force Space Test Program satellite, P78-1, since March 1979. Its broadband (4,100–6,350 Å) images show a Sun-centred annular field of view from 2.5 to 10.0 $R_\odot$ with a spatial resolution (pixel separation) of 1.25 arc min and with sensitivity adequate to record features having more than 10^{-10} times the brightness of the solar photosphere. In its normal operating mode, SOLWIND has obtained full-frame coronal images at 10-min intervals during the 1 h sunlit portion of each 97-min satellite orbit. This instrument and its initial observations have been discussed in detail elsewhere[1,7,8].

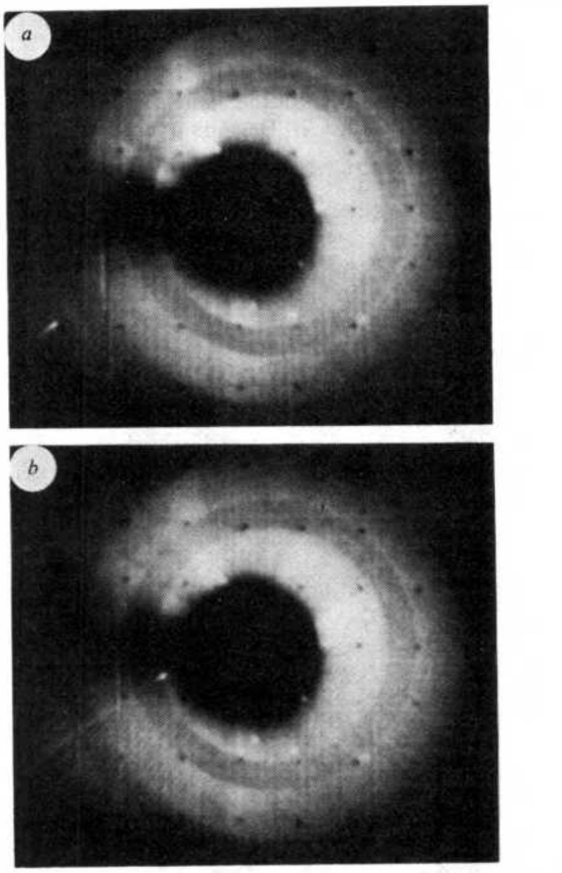

Fig. 1 The comet as seen against a background of the quiescent solar corona. *a*, The first frame in which the comet was detected, was recorded by the SOLWIND satellite coronagraph at 2039 UT on 26 January 1981. It shows the coma at a 7.9 $R_\odot$ projected distance from the Sun's centre. Subsequently, the coma dimmed noticeably (see Fig. 2 and discussion in the text), but it flared brightly again in *b* at 0020 UT 27 January 1981, just before passing behind the coronagraph's occulting disk, at 2.5 $R_\odot$. The dark, nearly concentric rings surrounding the occulter shadow are polarization analysers in the instrument's focal plane. Solar north is at the top in each frame, and east to the left.

In Fig. 1, the coronal images at 2039 UT 26 January 1981 and 0020 UT 27 January 1981 show the second of the three comets that we have observed with the SOLWIND coronagraph. The bright coma and fainter tail are visible as the comet approached the Sun from the south-east (lower left). In each image, the central dark area is the shadow of the instrument's occulting disk that artificially eclipses the Sun, and the dark region extending to the left is the shadow of the occulter support. The two dark, nearly concentric rings surrounding the occulter shadow are polarization analysers in the focal plane. They pass light that is polarized only in the radial direction, while the remaining regions pass light that is polarized only tangentially, thus favouring the Thomson-scattered light from free electrons in the Sun's *K*-corona. Note that the comet's light does not appear to be polarized, but that the bright coronal streamer in the north-east (upper left) is strongly blocked by the analyser rings.

We obtained 15 such images of this comet as it approached the Sun at a projected speed of 250 km s^{-1} before disappearing behind the occulting disk. Figure 2 shows a partial sequence of these images during 2039 UT 26 January–0030 UT 27 January 1981, and also includes an image at 0654 UT 27 January by which time the comet's nucleus should have reappeared from behind the occulting disk. In each image, the white disk was introduced to indicate the approximate size and location of the occulted photosphere. Also, unlike the images in Fig. 1,

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these images in Fig. 2 are difference images that show only intensity changes since 1300 UT on 26 January, before the comet's entrance into the field of view.

The temporal variation of the coma's apparent brightness is clearly visible in Fig. 2. Beginning around 2039 UT, the apparent brightness decreased steadily until 2351 UT after which it increased substantially as the comet came within a few radii of the Sun. Although we have not yet performed quantitative photometry, we think that the initial brightness decrease is a real effect and not simply the result of the radial decrease of the instrument's transmission function as one approaches the Sun. A comparison with stars and planets of known brightness suggests that the coma's brightness changed from a magnitude of roughly 0.0 at $8 R_{\odot}$ to $+1.0$ at $5.5 R_{\odot}$, and finally to -2.5 or brighter at $3 R_{\odot}$. Thus, unlike our first comet (1979 XI) whose magnitude reached approximately -3.5, this second comet was relatively faint during its initial approach to the Sun. This may be why it has not been reported previously despite the optimal position of its orbit relative to the Earth in the months before 26 January 1981. Of course, the fact that the comet did not reappear after perihelion accounts for why it was not observed in the months following 27 January 1981. Indeed, the sudden flare up of brightness around 0030 UT 27 January may indicate the beginning of the comet's final demise as it neared the Sun.

The 15 pre-occultation images were obtained during a scant 231-min interval with a spatial resolution (pixel separation) of 1.25 arc min and with modest absolute positional errors associated with image distortion and uncertainties in spacecraft roll angle. Consequently, it is difficult to determine the orbital elements uniquely, or even to distinguish between a retrograde orbit (motion counter to that of the planets around the Sun) or a so-called direct (prograde) orbit. Nevertheless, using the same technique that we applied to the observations of our first comet (1979 XI)[1], we have been able to find a small range of possible orbits (as a function of perihelion distance as a parameter) that fit the 15 pre-occultation observations within our

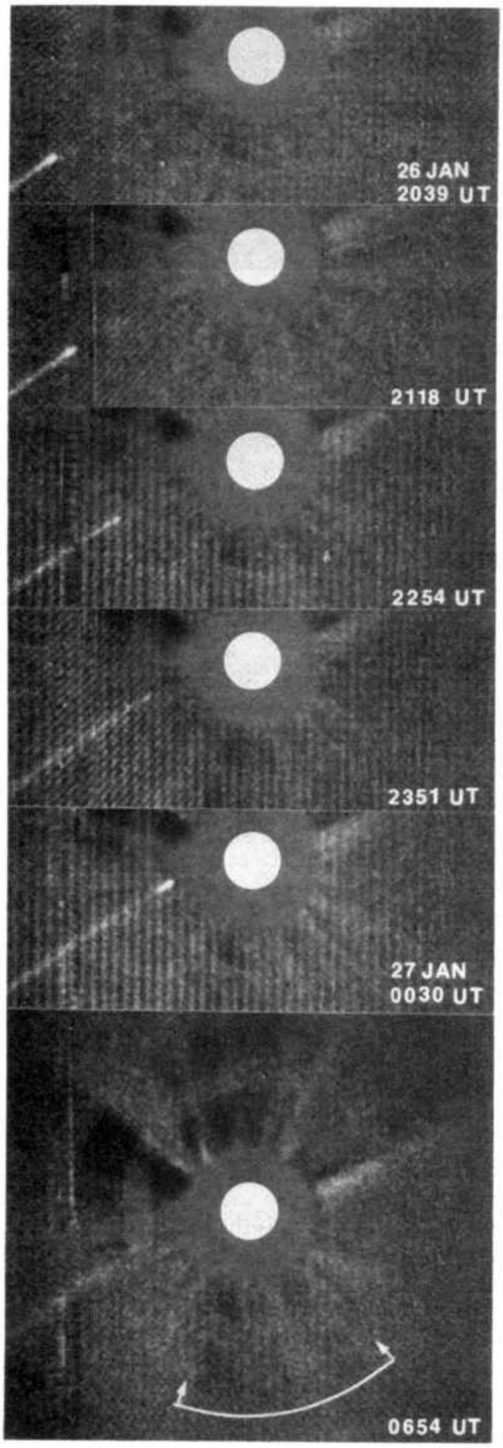

Fig. 2 Variations in the brightness of the coma are easily discernible in the first five frames, showing approach of the comet to perihelion. The sixth frame, after perihelion, shows a wider portion of the field at 0654 UT on 27 January. The arrows indicate a region in the corona through which the comet would have passed, had it survived perihelion, but no vestige of the coma was observed after the frame at 0030 UT 27 January. The photos in this sequence are difference images, showing only changes in the coronal scene after about 1300 UT on 26 January. A white disk has been added to each frame to indicate the location and size of the Sun, which is hidden by the coronagraph's occulting disk. The radial light and dark spoke-like features represent evolutionary increases and decreases, respectively, in the intensity of coronal streamers.

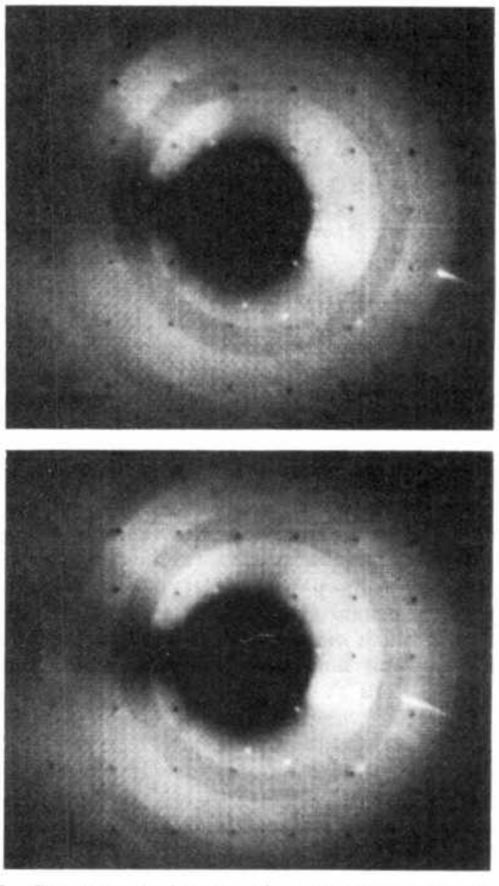

Fig. 3 Coronagraph images of a sungrazing comet as it approached the Sun from the south-west at 0209 UT and 0354 UT 20 July 1981. The format is the same as that in Fig. 1 with solar north approximately up and west approximately to the right. Unlike coronal streamers, whose polarized Thomson scattered emission is cut off by the dark polarizing rings, the comet is largely unpolarized and remains bright as it passes across the rings.

measurement accuracy. A retrograde orbit having a perihelion distance of $\sim 1.5\ R_\odot$, a perihelion time of 0230 UT 27 January, and orientation angles representative of the Kreutz group is consistent with our observations. [Marsden[9] has used our measurements to deduce a perihelion distance of $1.05\ R_\odot$, a perihelion time of 0149 UT 27 January 1981, and the orbital angular elements $\Omega = 350°$, $\omega = 72°$, and $i = 143°$ assuming that the comet's perihelion direction agrees with that of the Kreutz group.]

In Fig. 2, the area marked by arrows on the image at 0654 UT shows the region through which the comet would have moved outward from the Sun during the morning of 27 January 1981 if its perihelion distance lay in the range $1\text{–}2\ R_\odot$. Regardless of which of these representative orbits is assumed, the outbound coma should be visible in this image, but it is not. Nor was it visible on any other image obtained during 0001–1100 UT on 27 January 1981. Evidently, the comet either hit the Sun or was destroyed (or at least purged of its volatile material) in a close encounter. However, this does not explain why the 'outbound' tail was not visible after perihelion as it was with comet 1979 XI which we suppose hit the Sun[1]. At 0654 UT one can see only the fading trace of the tail along the inbound track together with some obvious coronal streamer changes, especially prominent in the Sun's northern hemisphere. It is possible that an 'outbound' tail, if it existed, would be undetectable for this relatively faint comet.

Figures 3 and 4 show similar observations of the third sungrazing comet that we have seen with the SOLWIND coronagraph. Figure 3 shows that the head of this comet is not cut off by the polarizing ring so that, like the first two comets, it is largely unpolarized. By comparison with a previously recorded image of the planet Mercury on 28 December 1980, we estimate that this third comet had a magnitude of -0.8 at $8\ R_\odot$.

More than 15 images were obtained before occultation on 20 July 1981, and numerous additional images were obtained during the subsequent day. The comet approached the Sun with a projected speed of $\sim 160\ \text{km s}^{-1}$ during 0029–0354 UT. As for the first two comets, our analysis of these images gave an orbit, which, if assumed to be retrograde, is consistent with those of the Kreutz group, this time with a perihelion of $\sim 1.2\ R_\odot$. Its perihelion time would have been 0805 UT 20 July, nearly the time of the fifth image in Fig. 4 in which the arrow marks the uneclipsed end of the comet's gradually lengthening tail. [Marsden[10] deduced a similar orbit with a perihelion distance of $0.9\ R_\odot$, a perihelion time of 0742 UT 20 July, 1981, and orbital angular elements $\Omega = 350°$, $\omega = 74°$, and $i = 143°$ again assuming that the perihelion direction agrees with that of the Kreutz group.] We suppose that the relatively short, tear-drop shape of this comet's tail is due in part to a perspective effect associated with the more oblique (head-on) orientation of the orbital axis relative to the line of sight than was obtained for the first two comets.

Finally, like our first two comets, this third sungrazer never reappeared after its occultation, and like the second comet (Fig. 1) no post-perihelion dust cloud was visible. In Fig. 4, the difference image at 2225 UT shows only coronal enhancements and depletions that have occurred in the 24 h since 2200 UT 19 July. The greatest enhancement lies in the south-east where the comet's head should have reappeared if its perihelion were in the range $1\text{–}2\ R_\odot$. However, this enhancement represents the accumulated evolution of a slowly brightening coronal structure whose initial growth is faintly visible in the earlier images in Fig. 4 well before this comet reached the Sun.

Perhaps the importance of these new observations is to suggest that sungrazing cometary encounters may be relatively frequent. Before the beginning of SOLWIND operation in March 1979, nine Kreutz sungrazers had been reported during the years 1668–1970. Since March 1979, we have seen three additional sungrazers despite the fact that we have processed coronal images for only 500 days to date (July 1982). Significantly, none of these three sungrazers was seen from the ground[11]. Evidently the SOLWIND coronagraph is observing

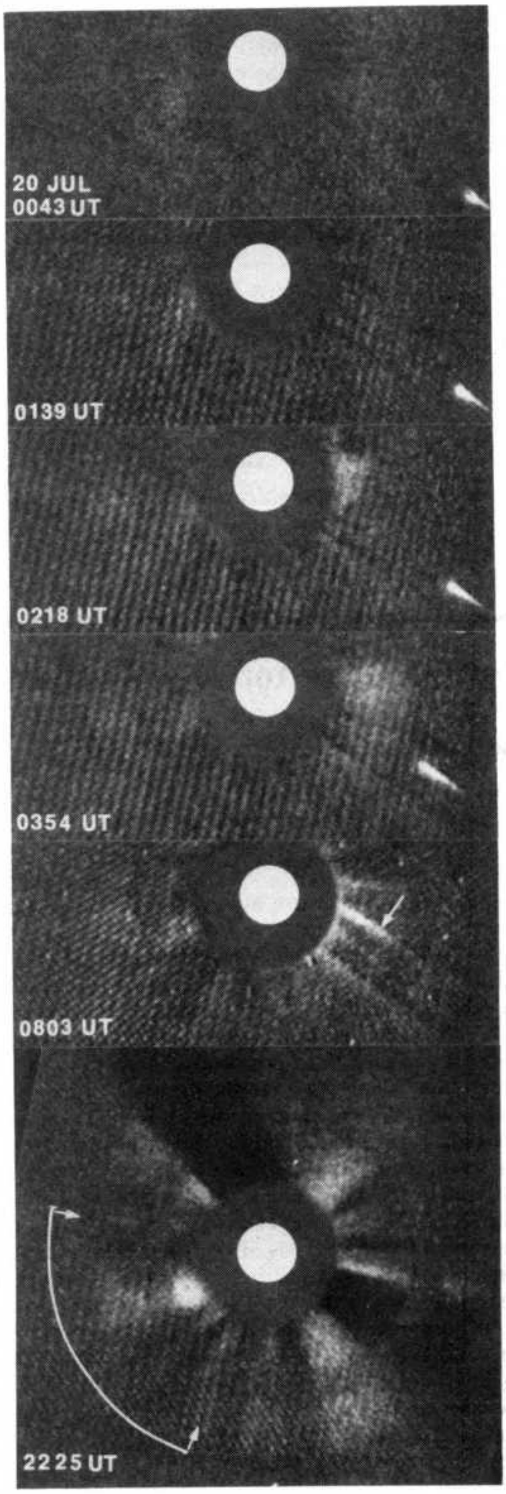

Fig. 4 A sequence of difference images showing intensity changes in the coronagraph field of view since 2200 UT 19 July 1981. Solar north is up and west is to the right. The inbound comet is clearly visible during 0043–0354 UT 20 July as it approached the Sun from the south-west. Its tail is still visible at 0803 UT (arrow) by which time the head has passed behind the occulting disk. By 2225 UT, there is no trace of either head or tail among the numerous increases and decreases of coronal intensity. The greatest enhancement, visible in the south-east where the comet's head is expected to reappear (arrows), represents the accumulated brightness increase of a coronal structure whose initial brightening is already faintly visible in the pre-occultation frames of this figure. The white disk at the centre of each image indicates the apparent size and location of the occulted photosphere.

the faintest and perhaps most perishable fragments of the supposed primordial comet whose close encounter with the Sun may have led to the formation of the Kreutz group.

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A dispersion law for solar oscillations

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The pressure or *p*-modes are acoustic vibrations trapped in a resonant cavity below the solar surface. The waves are most easily observed as primarily vertical velocities at the solar surface by Doppler shift techniques. The vertical velocities vary harmonically in time and in space across the solar surface. These harmonic variations lead to a natural description of the oscillation modes in terms of their positions in a diagram of temporal frequency (ω) versus horizontal spatial frequency (k_h). Observationally, the positions in the k_h–ω diagram are determined from a two-dimensional power spectrum of a series of Doppler shifts of a solar spectrum line measured equidistant in space and time. An example of the observed positions is shown in Fig. 1 (see ref. 1). Theoretically, the mode positions are determined by an analysis of the solar cavity, including an uncertain model of the solar interior. Most theoretical work has consisted of numerical solutions of the equations of motion leading to predicted positions in the k_h–ω diagram (see, for instance, ref. 2). This approach has been reasonably successful, yielding inferences about the depth of the convection zone[3]. Here we compare a simple model of the oscillations discussed by Leibacher and Stein[4] with observations.

In the Leibacher and Stein[4] model the resonant cavity is a spherical shell. To first order the outer boundary of the shell is considered to be the same for all modes and is defined by the sharp decline in the density scale height just below the visible surface. The inner boundary of the shell is caused by the increase of sound speed with depth. Waves travelling downwards at an oblique angle to the vertical (with non-zero horizontal wavenumber k_h) are refracted away from the vertical by the increasing sound speed. At some depth the propagation becomes horizontal and the wave is reflected, this marking the lower boundary of the cavity. At this depth the sound speed is equal to the horizontal phase velocity ω/k_h. Leibacher and Stein[3] argue that the boundary conditions should be similar to an organ pipe closed at one end and open at the other. The WKB approximation is then used to derive the condition: $(n+1/2)\pi/\omega = \int dz/c$. Where z is the vertical coordinate, c is the speed of sound and n is the number of nodes in the vertical standing wave patterns. The integral is over the depth of the cavity. $\int dz/c$ is the travel time of sound across the cavity. The constant 1/2 depends on the choice of boundary conditions[5]. The different curves in Fig. 1 correspond to different values of n while distance along a given curve corresponds to cavities of different depths.

From this simple model we draw two conclusions. (1) If the top of the cavity is the same for all modes and the bottom of the cavity is defined by the position where $c = \omega/k_h$, then modes in different parts of the k_h–ω diagram with the same value of ω/k_h are trapped in an identical cavity. (2) If the cavity is identical, the travel time of sound across the cavity will be the same for different modes. Therefore, in a plot of ω/k_h (an observed quantity) versus $(n+1/2)\pi/\omega$ (another observed quantity), the ridge structure of Fig. 1 should collapse onto a single curve. This leads to a modified dispersion law of the form $(n+1/2)\pi/\omega = f(\omega/k_h)$.

Using this procedure we found that the ridge structure did not collapse onto a single curve. To admit the possibility of other boundary conditions similar plots were constructed, but with the ordinate given by $(n+\alpha)\pi/\omega$, for different values of α. It was found that near $\alpha = 3/2$ the points collapse onto a single curve, within the observational accuracy (Fig. 2). To determine a best value for α the residual variances to fifth-order polynomial fits to the resulting points was computed as a function of α. The minimum residual variance (or the tightest curve) was found for the value $\alpha = 1.50$. The standard deviation of the points about the polynomial fit is 1% of the mean value of $(n+3/2)\pi/\omega$. The fundamental mode ($n = 0$) has been excluded from this analysis and from Fig. 2 as its frequencies do not fall along the curve of Fig. 2.

An important aspect of the above result is the reduction of the redundancy in the k_h–ω diagram. Essentially all of the information about the solar interior contained in the multiple curves of Fig. 1 is concentrated in the single curve of Fig. 2. Modes trapped in an identical cavity are giving redundant information, and the result of Fig. 2 strongly suggests that modes with the same value of ω/k_h are trapped in an identical cavity. This is surprising, as the assumption of an identical boundary at the top for all modes is really a gross simplification of the problem. The upper boundary should really depend on

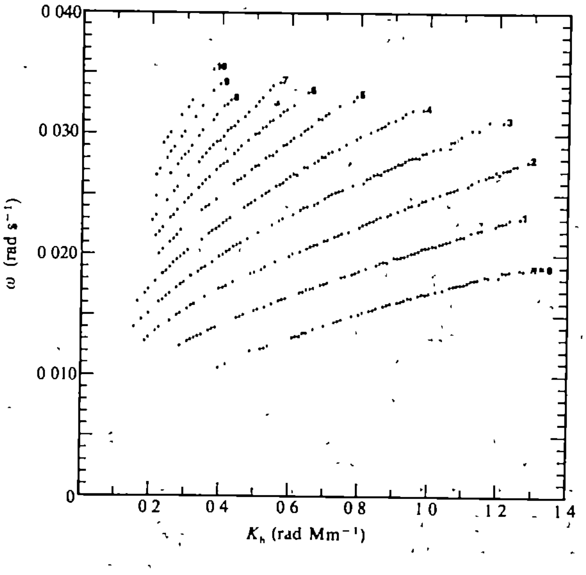

Fig. 1 Mode frequencies derived by a centroid analysis from a two-dimensional (ω, k_h) power spectrum of velocity observations made at Kitt Peak in May 1980 (ref. 1). The curves are labelled with the radial harmonic number n. Solar rotation has been cancelled by averaging the frequencies ω for eastward and westward propagating waves.

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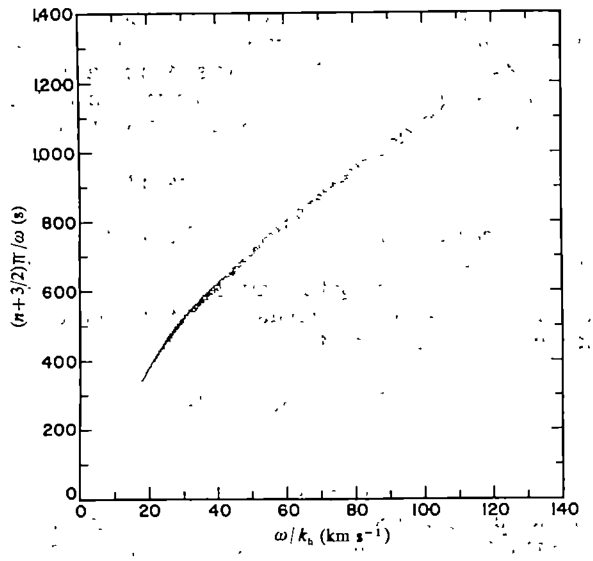

Fig. 2 Plot of $(n+3/2)\pi/\omega$ against ω/k_h for the mode positions of Fig. 1. The $n = 0$ modes are excluded.

ω (ref. 6). The above result suggests that the difference in boundary conditions at the top for different modes has a minimal effect on the resulting frequencies.

To within the observational accuracy the best value of α is 3/2. This may be fortuitous, but it does admit the possibility that the Leibacher and Stein analysis is correct and that we have misidentified the radial node number n. This seems unlikely because of the general agreement between the theory and observed frequencies. However, n is not observed directly but is inferred from this general agreement. So n misidentification is possible though unlikely.

Another possible source of the value $\alpha = 1.50$ is the atmospheric structure. Christensen-Dalsgaard[7] has shown that for a polytrope of index m the frequencies obey a relation $\omega/k \propto [(n+m/2)/\omega]f(n, m)$, where $f(n, m)$ has a weak dependence on n and a dependence on other atmospheric structure parameters. If we could assign a polytropic index $m = 3$ for the solar convection zone, we could approach the result of Fig. 2. However, the polytropic index is a strong function of position in the upper convection zone according to current models. Also, the function $f(n, m)$ for $m = 3$ introduces differences between modes of different n that are larger than the observed scatter in Fig. 2. Although the polytropic model may not be directly applicable, it does point out a way of getting a dispersion law that is dependent on the atmospheric structure.

We have considered whether the data might be better fitted by an α which is a function of another parameter, possibly ω/k_h or ω. However, the data are fitted to the observational accuracy by a constant α and so we have not felt justified in introducing further dependencies.

If the ordinate of Fig. 2 is the travel time of sound across the cavity and the abscissa the speed of sound at the bottom of the cavity, the data of Fig. 2 could be directly inverted to obtain the speed of sound plotted against depth. We are investigating this possibility.

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Critical density fluctuations within a single polymer chain

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The coil–globule phase transition is a reversible, conformational change of a macromolecule where the high temperature (or good solvent) phase is an extended coil and the low temperature (or poor solvent) phase is a tightly packed globule[1]. We now present direct observations, made by photon-correlation spectroscopy, of the sharp increase in amplitude and relaxation time of density fluctuations within a single polymer molecule as it passes through the coil–globule phase transition. This growth of fluctuations is characteristic of critical phenomena associated with phase transitions, a subject that has generated much interest over the past decade, and has been studied in a wide variety of physical systems. These are the first measurements of critical behaviour that we know of made on a three-dimensional system that can be characterized as finite in the terms of statistical mechanics.

The phase transition is a manifestation of competition between two forces acting on the polymer chain. Affinity among polymer segments tends to shrink the polymer, while an entropic pressure originating from the thermal motion of the individual segments tends to expand it. At higher temperatures

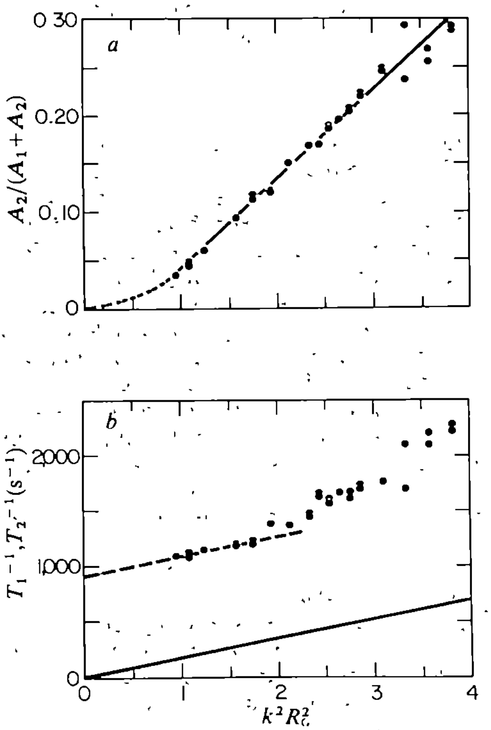

Fig. 1 Characteristics of intramolecular fluctuations at 33.9 °C plotted as a function of the parameter $x = k^2R_G^2$. *a*, The normalized amplitude. *b*, The frequency of internal motion. The solid line is the frequency associated with the translational diffusion. Points representing the frequency of the lowest order mode of internal motion fall parallel to the solid line at small values of x.

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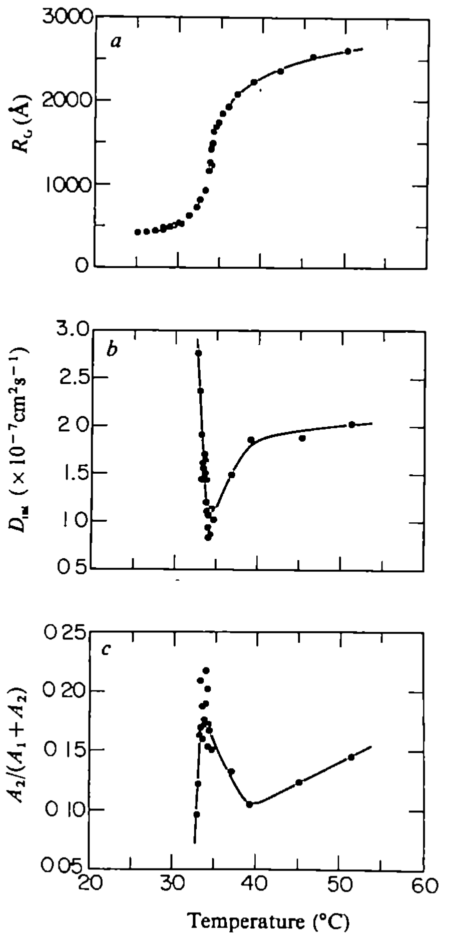

Fig. 2 The behaviour of the intramolecular fluctuations as a function of temperature (lines to guide the eye). *a*, The collapse seen in the radius of gyration. *b*, The internal diffusivity of the segments, $D_{int}=R_G^2(T_2^{-1}-T_1^{-1})$. *c*, The amplitude of the fluctuations at $x = 2.5$.

(or in a good solvent) the entropic pressure dominates the segment affinity, and the polymer is expanded. At lower temperatures (or in a poor solvent) the segment affinity is dominant, and the polymer collapses. For flexible polymers of large molecular weight the collapse is quite drastic[2-4]. The polymer conformation is not frozen in during the process of collapse, however. Individual segments along the polymer backbone undergo thermal motion resulting in intramolecular concentration fluctuations. The brownian diffusion of the macromolecules as a whole through the solvent also creates concentration fluctuations, but on a longer time and length scale. The autocorrelation function of light scattered from the polymer solution contains information from both sources. The amplitude and relaxation time of intramolecular fluctuations can be determined by careful analysis of the correlation function at various scattering angles.

The polymer–solvent system used in the experiment is polystyrene (Polysciences $\bar{M}_W=2.6\times10^7$, $\bar{M}_W/\bar{M}_N=1.3$) in cyclohexane ($T_\theta = 35.4$ °C). The light scattering apparatus is of typical construction. The sample solution's temperature stability could be maintained to within a couple of mK over a period of several days. This is especially important in the region of the transition, where polymer size changes drastically with temperature. The sample was sufficiently dilute ($\sim10^{-6}$ g ml^{-1}) so that the collapsing polymers would remain dispersed without aggregation.

Different length scales can be probed by varying the angle of collection of photons scattered by the polymer solution. The length scale is determined by the inverse of the scattering vector, $k=(4\pi/\lambda)\sin(\theta/2)$, where λ is the incident wavelength in the solution, and θ is the scattering angle. A useful parameter for characterizing the length scale is $x=k^2R_G^2$, where R_G is the polymer radius of gyration. At forward scattering angles where $x<1$, only length scales larger than the molecular size are probed; the correlation function is a single exponential,

$$g^{(2)}(t)\propto\exp(-2Dk^2t)+\text{constant}$$

where D is the translational diffusion coefficient of the polymer. Shorter distances are probed in the range $1<x<4$. The lowest internal mode representing fluctuations in the overall polymer size contributes to the correlation function, which is now described by two components,

$$g^{(2)}(t)\propto\exp(-2Dk^2t)\{P_0(x)+P_1(x)\exp(-t/\tau)\}^2$$
$$+\text{constant}$$

where τ is the relaxation time of the lowest mode[5,6].

An established procedure was used to characterize the internal motion[7,8]. At each temperature, correlation functions were measured at forward scattering angles ($x<1$) to determine D. The correlation function was then measured at the higher scattering angles and fit to

$$c(t)=\{A_1\exp(t/T_1)+A_2\exp(t/T_2)\}^2+B$$

where T_1 is fixed by D. The parameters A_1, A_2, T_1, and B were varied.

Figure 1*a* shows the behaviour of the normalized amplitude of the internal motion, $A_2/(A_1+A_2)$, as a function of x at 33.9 °C. Figure 1*b* shows the inverse relaxation time, T_2^{-1}, also as a function of x at the same temperature. The translational relaxation time T_1^{-1} is plotted as a straight line through the origin in Fig. 1*b*. The value of τ is obtained from the difference in T_1^{-1} and T_2^{-1} in the region where they are parallel. For higher values of x, the effect of the higher-order modes corresponding to shorter length-scale density fluctuations is apparent—the two-exponential form is no longer appropriate to describe the measured correlation function. Figure 2*a* reproduces our previously published results showing the collapse in the radius of gyration as temperature is lowered[4]. Figure 2*b* is a plot of the diffusivity of the internal motion defined as $D_{int}=R_G^2/\tau$, derived from the relaxation time of the intramolecular fluctuations versus temperature. This combination of R_G and τ is appropriate since τ scales in proportion to R_G^2, and because the proportionality coefficient D_{int} actually represents the intramolecular segment diffusivity[5,9]. The slowing intramolecular motion is quite apparent in this plot. Figure 2*c* is the normalized amplitude $A_2/(A_1+A_2)$ versus temperature at $x=2.5$, indicating an approach to critical divergence.

More precise measurements of this phenomenon are underway. Future experiments that use carefully prepared monodisperse polymers in a range of molecular weights to measure the effect of the size of the statistical system on the critical behaviour would prove interesting. The physics of the polymer conformational fluctuations in the vicinity of the collapse phase transition may also prove useful in describing the function of certain biological macromolecules.

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Meandering of the subtropical front south-east of the Azores

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A subtropical front was observed in the area south and south-east of the Azores during cruises of FS *Meteor* and FS *Poseidon* in early 1982. The front has a basically west–east extension, with considerable meandering observed. Meso-scale eddies are found on both sides. The overall flow pattern corresponds to earlier results on geopotential differences in the upper north-east Atlantic, but the baroclinic transport of the order of $10^7\ m^3\ s^{-1}$ is found to be concentrated in a 60-km wide jet. We suggest here that the current band is part of the gyre circulation, resulting from a branching of the North Atlantic Current.

Such subtropical fronts can be expected where strong Ekman transport convergence occurs in a region of large horizontal thermohaline or haline gradients. They are temperature and salinity fronts in winter and spring and salinity fronts only in summer and autumn in the North Pacific[1], with a density front existing simultaneously at both times of the year. Jet-like baroclinic surface currents have been found at a 1,000-km long front in the central subtropical North Atlantic, with current speeds as high as 50 cm s^{-1} (ref. 2). The subtropical front is more pronounced in the Southern Hemisphere, and a complicated mesoscale structure connected to the front has been found in the southern Indian Ocean[3].

The subtropical front also marks the transition between the eastward and westward flow in the oceanwide anticyclonic gyre circulation. Early studies of the North Atlantic circulation in the area north of the Azores[4,6] led to the conclusion that the North Atlantic Current splits into two branches at approximately 45° N, 25° W, with a southern branch heading towards the Azores and continuing south-east towards Madeira. The upper ocean flow field can most easily be seen in the potential density field in Fig. 77 of ref. 6, indicating a splitting-up into two more branches west of the Azores. An eastern branch in this historical map flows southward to the Azores, then veers left towards the south-east. A second branch reaches the Azores west of the Mid-Atlantic Ridge, oriented west at first at 37° N and turns back to the east at 35° N. Note that Hansen's[5] numerical model of Atlantic Ocean surface currents including a variable wind field produced a dynamic topography that is consistent with a flow from the Azores to the Madeira region. A similar basic pattern can be found in more recent maps of upper ocean geopotential differences[7–9].

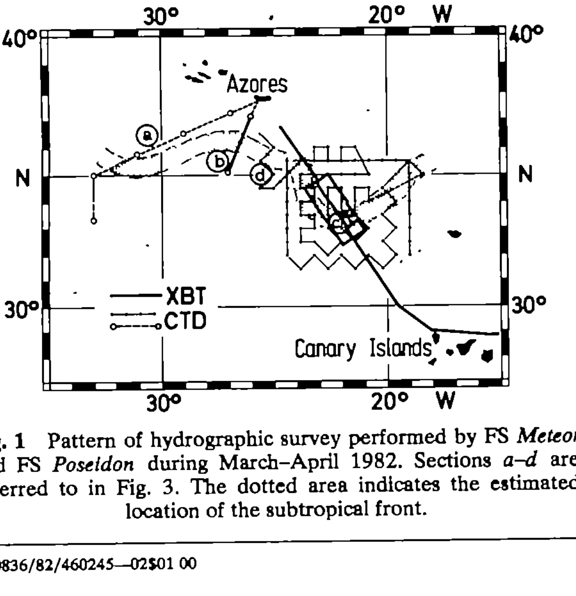

Fig. 1 Pattern of hydrographic survey performed by FS *Meteor* and FS *Poseidon* during March–April 1982. Sections *a–d* are referred to in Fig. 3. The dotted area indicates the estimated location of the subtropical front.

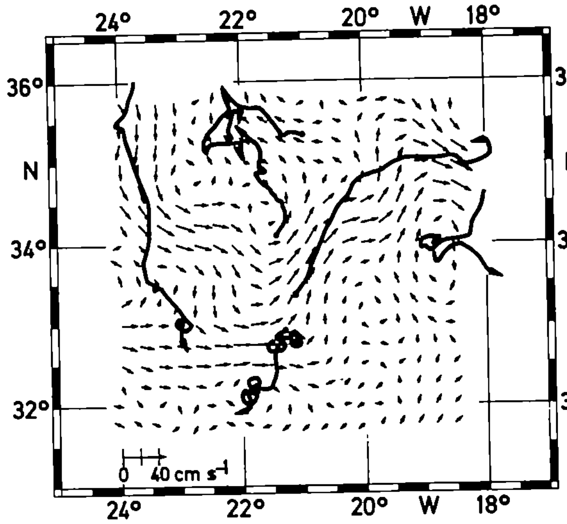

Fig. 2 Estimated geostrophic surface velocity relative to 1,500 dbar and paths of satellite-tracked drift buoys with drogue at 100 m depth. Map determined from objective analysis of geostrophic surface velocity relative to 1,500 dbar using a non-isotropic gaussian covariance function with zonal and meridional correlation scale of 100 and 65 km. Trajectories of satellite drift buoys obtained from daily fixes during FS *Poseidon* hydrographic observations.

These early data led to an easterly flow in the region where Ekman transports calculated from Bunker's[10] wind data (H.-J. Isemer, personal communication) result in an Ekman convergence zone approximately along 35° N, with a seasonal meridional shift of ±3° latitude. These results on the gyre circulation and the Ekman convergence suggest a connection between the current branching from the North Atlantic Current system towards the Azores and the current related to the subtropical front in the eastern North Atlantic.

Within the framework of the Warmwassersphäre research programme at Kiel University several cruises were performed in the tropical, subtropical and moderate latitude basins of the eastern North Atlantic. Indications of frontal variability were found to exist persistently in the region between 30 and 38° N, and subsequently observational programmes were designed for 1982 cruises with the aim of resolving mesoscale features of the frontal region south and east of the Azores (Fig. 1). FS *Meteor* in March/April occupied several XBT and CTD sections including a XBT box pattern between the Canary Islands and the Azores in the northern Canaries Basin and also south-west of the Azores close to Mid-Atlantic Ridge. FS *Poseidon* in April covered 82 CTD stations 30 nautical miles apart in a box pattern between the Azores and the Canary Islands. Also four satellite buoys deployed by FS *Meteor* previously were drifting in the area of the FS *Poseidon* box.

The geostrophic flow field obtained from the CTD box data is presented in Fig. 2. It reveals a current band with ~60 km width and 25 cm s^{-1} speed in its core, entering the box in the northwestern corner, turning east in the centre and leaving the area from the northeastern corner. This current band corresponds to large horizontal temperature gradients and also to salinity gradients in the upper ocean that specify a front. The related geostrophic shear reaches levels as deep as 650 m, more than is usually reported for surface fronts. The satellite drifters' tracks also shown in Fig. 2 conform with the general pattern of geostrophic currents obtained from the CTD data. Note that closed streamline patterns on both sides of the jet suggest the presence of mesoscale eddies with typical scales of 200 km.

The larger-scale spatial distribution of the front can be determined from further surface temperature data and temperature sections to the west of the CTD box (see Fig. 1). The surface temperatures displayed in Fig. 3 include the position of the front marked by the maximum horizontal gradient at the surface. The corresponding temperature sections are also shown

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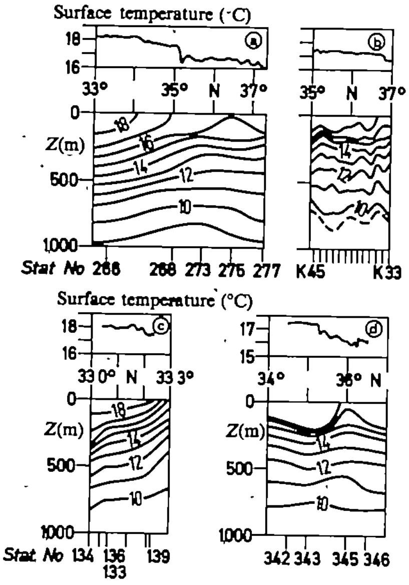

Fig. 3 Temperature obtained from sections *a–d* indicated in Fig. 1. Note the different scale in section *c*.

in Fig. 3. The front stretches zonally south of the Azores, turns to a southerly direction south-east of the Azores and in the box veers round to northerly directions. IR radiation satellite photographs confirm the existence of a continuous temperature gradient band in this general pattern (G. Hardtke, personal communication). Time variability of the front on time scales of the order of 1 month can be deduced from current meter moorings in the area. This will be discussed elsewhere.

The long-term persistence of the front can be tested by inspecting data from three different cruises: RRS *Discovery*, January 1981[11,12], FS *Meteor*, April 1981, and FS *Poseidon*, April 1982. The density at 250 m depth obtained in sections crossing the area under discussion is presented in Fig. 4. The horizontal density gradient related to the front is found at similar positions in all three sections. The observed surface front obviously separates regions with a permanent thermohaline mixed layer and subtropical thermocline from regions with seasonal thermocline formation, winter convection and advection of North Atlantic Current water masses. We suggest that the subtropical front found here corresponds to a branch of the North Atlantic Current that is already indicated as a broad band in Wüst's[6] map of potential density at 200 m. This is supported by the fact that downstream volume transports in the observed baroclinic current band related to the front are of the same order of magnitude (10^7 m^3 s^{-1}) as mean meridional Sverdrup transports across this area between the Mid-Atlantic Ridge and the African coast estimated from zonal hydrographic sections[11,12]. Transports in the narrow band at the front may thus constitute the major portion of the total long-term transport. It is also possible that the front observed here is a continuation of the frontal zone found[13] to the west of this area.

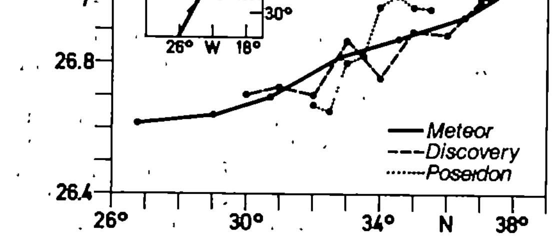

Fig. 4 Density (σ_T) at 250 m depth as function of latitude from three different cruises in the area south-east of the Azores.

The observed meandering of the front, with an even more complicated smaller-scale structure seen in IR satellite photographs, may be due to several processes. The most obvious candidates are quasi-stationary Rossby waves, which were earlier suggested[14] as the cause of wave-like patterns observed in the polar boundary of the subtropical convergence in the South Atlantic, or baroclinic instability of the jet.

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Oxygen utilization rates in North Atlantic subtropical gyre and primary production in oligotrophic systems

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If the observed distribution of dissolved oxygen in deep oceans below the euphotic zone is in stationary and steady state, then it can be assumed that the *in situ* consumption of oxygen is balanced by 'ventilation', or, in other words, physical transport of oxygen from regions of higher dissolved oxygen and ultimately the sea surface. It follows, therefore, that measurement or determination of those physical transport processes, coupled with observation of the oxygen distribution will lead to an estimate of the net oxygen utilization rate (OUR). The character of such an estimate is that it is a space and time average over scales governed by the nature (space and time scales) of the transport processes, the scale of the oxygen distribution, and the climatologic nature of the system. As such, one obtains an average which is characteristically of the order of 10^8 s, 10^8 cm or more, that is large compared with scales of biological variability. I report here some new determinations of oxygen utilization rates determined by tritium dating in the North Atlantic subtropical gyre, compare them with other and previous estimates, and discuss their implications regarding primary production and nutrient cycling in an oligotrophic system.

The Beta Triangle is a triangular study area in the eastern subtropical North Atlantic whose apices are at 26.5° N × 38.5° W, 32.5° N × 30.0° W, and 22.5° N × 28.5° W. The area was chosen as a testing ground for the Beta–Spiral calculations—a geostrophic computation which yields absolute flow velocities[1]. The Triangle has sides of ~1,000 km each and is characterized by significant oxygen gradients on isopycnals. The shallow flow is of the order of 1 cm s^{-1} to the south-west.

As part of the Beta–Spiral study, the Triangle was intensively sampled for the transient tracers tritium (^3H) and ^3He. The strategy was to use the observed tracer distributions to obtain an independent estimate of velocity field for comparison with the dynamical (Beta–Spiral) calculations. The preliminary results agree well (work in preparation) which lends credence to the results obtained using the tracers alone.

Tracer samples were obtained on three density levels ($\sigma_\theta =$ 26.45, 26.6, 26.8‰) at 15 stations on the perimeter of the Triangle and three stations in the interior. The tritium and ^3He were measured using the mass spectrometric method[2] to 1.0% (±0.04 TU) and 1.6‰ respectively, and the ^3H–^3He age computed as

$$T = 1/\lambda \ln\left(1+\frac{[^3He]}{[^3H]}\right) \qquad (1)$$

where $1/\lambda$ is the tritium mean life, here taken as 17.96 yr. Age non-linearity is not significant on the time scales observed here[2], particularly because the tritium field is homogeneous on these levels.

The data are summarized in Fig. 1, where the regression lines obtained yield the OUR for each surface. Note that the ^3H–^3He age range encompassed by each surface is of the order of several years; that is, an order of magnitude greater than measurement uncertainty. The same holds true for the oxygen range. The least squares regression parameters are summarized in Table 1 with the uncertainties (1 s.d.) of the estimates. It is encouraging that the intercepts, the zero age AOU values, are not significantly different from zero.

Implicit in this analysis is the assumption that lateral mixing is small. Armi and Stommel[3] have analysed the salinity fields in the Triangle and their results confirm this hypothesis. Furthermore, lateral mixing will, in the first order, be transparent as it will reduce not only the ^3H–^3He age gradients, but also the oxygen gradients. At least for the present analysis, therefore, it is reasonable to adopt the statistical uncertainties in the regression slopes as representative of the accuracy of this OUR determination. These are reflected in the error bars for these OUR measurements in Fig. 2.

Riley[4] used a geostrophically 'calibrated' three-dimensional advection diffusion calculation for the North Atlantic to obtain estimates of OUR for various surfaces. Inasmuch as uncertainty in the reference level for the geostrophic calculations produces a barotropic uncertainty no more than the order of a few millimetres per second, the shallow estimates are probably accurate to within 10–20%, whereas deeper estimates may be more suspect. Those results, plotted on a depth scale, are compared with the present results in Fig. 2.

Figure 2 also shows results obtained from ^3H–^3He dating[5] and from a ^3H–^3He calibrated lateral mixing model[2], both in the Sargasso Sea. These calculations differ from the Beta Triangle OUR values in that they are 'end-point' determinations—they reference to saturation values for oxygen at zero ^3H–^3He ages. The Beta Triangle OUR, on the other hand, represents a gradient determination.

The results all show a remarkable consistency for depths above 1 km, particularly in light of the differences in the techniques. Although the results diverge below 1 km, this can be explained on the basis of the barotropic uncertainty in Riley's computations[2]. Irrespective of this disparity, the bulk of the water column oxygen utilization occurs above 1 km where the results agree.

The oxygen utilization rates shown in Fig. 2 below the euphotic zone (~100 m in depth) may be integrated to yield the net oxygen consumed in the water column. The simplest approach is to use a log linear consumption rate–depth dependence, the best fit being

$$\ln \mathrm{OUR} = -(0.68 \pm 0.17) - (0.00295 \pm 0.00027)z \qquad (2)$$

where OUR is the oxygen utilization rate in ml per l per yr and z is the depth in m (positive downward). Integrating between 100 m and infinity, one obtains 5.7 mol oxygen consumed m^{-2} yr^{-1}. In order that the consumption of this oxygen be supported, the water column below the euphotic zone must be supplied with ~4.5 mol C m^{-2} yr^{-1}, or 55 ± 5 gC m^{-2} yr^{-1}.

This corresponds to a remarkably large flux of carbon out of the euphotic zone. It is double the currently accepted mean oligotrophic primary production rate[6], and if the level of recycling of primary production carbon approaches 80–90% as recently suggested[7,8], then the actual amount of carbon fixed at the ocean surface in the subtropical gyre may be several hundred grammes of C m^{-2} yr^{-1}. This result, therefore, may conflict with commonly accepted ^{14}C assimilation rate measurements of net surface primary production.

Schulenberger and Reid[9] used observations of shallow subsurface oxygen maxima to estimate minimum surface oxygen production and hence carbon fixation rates. Their results showed carbon fixation rates 2 to 7 times higher than ^{14}C assimilation estimates, and because that they could not account for what may be substantial photosynthetic oxygen loss to the atmosphere, represented a conservative lower limit to the disagreement between the techniques.

Primary production estimates have been based on the small bottle, long incubation technique of ^{14}C-bicarbonate assimilation. This technique has recently been criticized by workers[9–11] who have suggested that it may substantially underestimate the true carbon uptake. Whereas the ^{14}C uptake technique may suffer from statistical sampling problems (due to space and time variabilities) and the possible difficulty of perturbing the systems being measured, the water mass tracer techniques achieve realistic space–time averages and are true *in situ* determinations. The results described here represent time averages extending from years to decades (in the deeper waters) and space averages from a few hundred kilometres to the whole subtropical gyre. The Schulenberger and Reid results[9] yield time averages of a few months and space averages probably of the order of a few hundred kilometres. Clearly the water mass approach, which is based on a wide variety of techniques—transient tracers, dynamical calculations and seasonal photosynthetic oxygen cycles—presents a compelling argument for either substantially higher surface production than depicted by the ^{14}C assimilation techniques, or less efficient recycling within the euphotic zone. Whereas the results presented here only constrain the flux out of the euphotic zone, other results[9–11] suggest that at least part of the problem lies with the ^{14}C techniques.

The proposed large surface productivity requires a correspondingly greater supply of nutrients (NO_3 and PO_4). The requirement is that the flux of carbon from the euphotic zone be balanced in the appropriate stoichiometric proportions by a return of nitrogen and phosphorus from the deep water. Using a transient tracer calibrated lateral transport model[2], one can

Table 1 AOU–^3H–^3He age regression results

Surface (σ_θ in ‰)	Approximate depth (m)	Intercept (ml per l)	Slope (OUR) (ml per l per yr)
26.45	200	0.10±0.08	0.173±0.029
26.60	300	−0.10±0.11	0.196±0.025
26.80	400	0.12±0.18	0.169±0.028

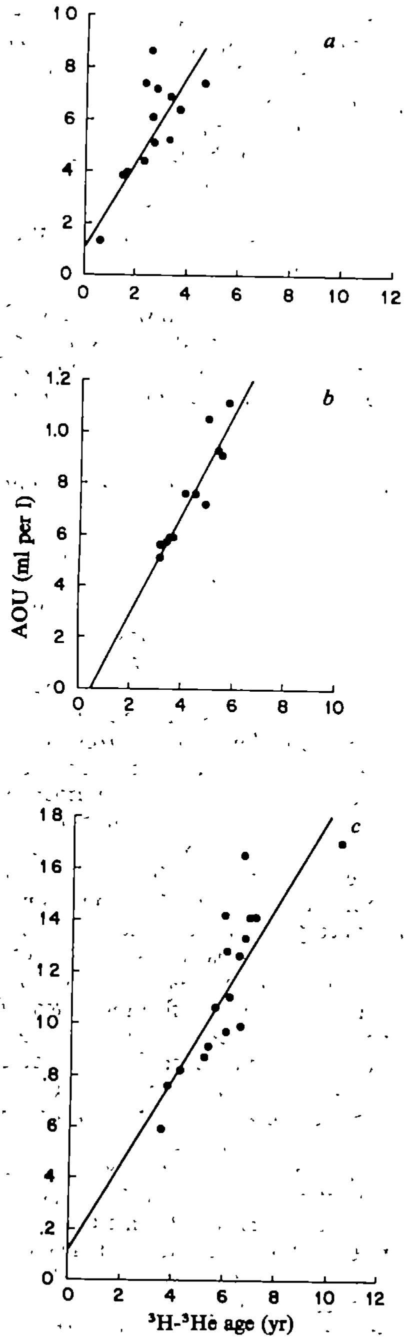

Fig. 1 AOU plotted against ^{3}H–^{3}He age for three density surfaces *a*, $\sigma_\theta = 26.45$; *b*, $\sigma_\theta = 26.60$; *c*, $\sigma_\theta = 26.80$ in the Beta Triangle (see text).

estimate the reflux of for example, nitrate to the surface by the relation

$$F(\mathrm{NO_3}) = \int_{100}^{D} \frac{C(\mathrm{NO_3})}{T}\,\mathrm{d}z \qquad (3)$$

where $F(\mathrm{NO_3})$ is the flux of nitrate, $C(\mathrm{NO_3})$ is the depth-dependent water column nitrate concentration and T is the depth-dependent residence time[2]. The integration is from some depth D to the base of the euphotic zone (here assumed to be 100 m).

Using GEOSECS station 121 (35°59′ N, 67°59′ W) nitrate data[12] as a typical Sargasso Sea nitrate profile and integrating as in equation (3), one obtains a nitrate flux of 0.7 mol $\mathrm{NO_3}$ $\mathrm{m^{-2}\,yr^{-1}}$ which almost perfectly matches the requirements of the carbon flux reported here. A similar calculation may be performed for phosphate, but note that inasmuch as the Redfield ratios for AOU : N : P are observed quantities, and that the nutrient fluxes are 'calibrated' using the same tracer model as the OUR estimates, the nutrient fluxes must *a priori* yield a result consistent with the carbon flux.

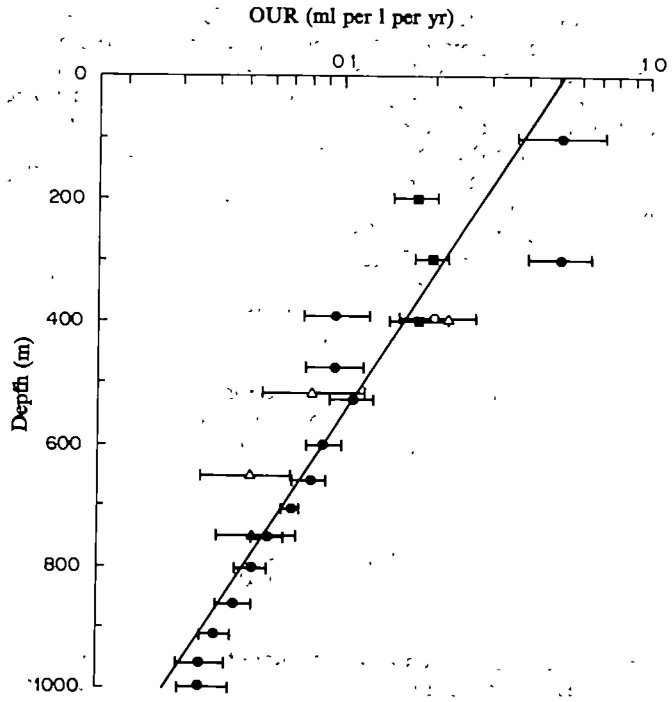

Fig. 2 Oxygen utilization rates plotted against depth from ^{3}H–^{3}He dating (■, ○), a geostrophically calibrated three-dimensional advection–diffusion model (△) and a ^{3}H, ^{3}He calibrated isopycnal mixing model (●).

On the basis of large scale tracer distributions, dynamical balance and subsurface seasonal photosynthetic oxygen maxima, I conclude that surface fixation of carbon in the subtropical open ocean is many times greater than deduced from ^{14}C assimilation techniques. A downwards carbon flux from the euphotic zone in excess of 50 g C $\mathrm{m^{-2}\,yr^{-1}}$ is deduced from water column OURs. These essentially independent determinations, based on different approaches are all characterized by large space and time scale averaging. This, coupled with the fact that they are independent yet yield the same results lends credence to the high rates predicted. It is therefore becoming increasingly clear that the ^{14}C assimilation techniques are in error and that oceanic 'oligotrophic' primary production is considerably higher than previously thought.

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Amino acid evidence for Devensian ice, west Gower, South Wales

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Complete inundation of South Wales by ice during the penultimate (Wolstonian?) glaciation has been widely suggested[1-3]. Although it is generally agreed that the last glaciation was less extensive than the preceding one[4-6], there has been controversy concerning its limits, stemming mainly from the lack of clear geomorphological evidence and the dearth of datable material[7-9]. ^{14}C dates from the shelly till in Pembrokeshire were first thought to indicate an extension of last glaciation ice into the area[10], but serious doubts were subsequently raised about the reliability of finite dates on shells of this age range[11,12]. We present here results of amino acid analysis of shells from recently exposed till and from underlying raised beach remnants at Broughton Bay, west Gower. Total D *allo* : L *iso* ratios from the raised beach are consistent with analyses on shells from comparative beach remnants elsewhere in south-west Britain[7,13-15]. Shells of 21 identifiable littoral species extracted from the till suggest inshore marine conditions similar to those at present. Amino acid ratios from *Littorina* spp. provide firm evidence for the encroachment of Devensian ice on to Gower, approximately to the limit suggested by Bowen[7] (Fig. 1*a*).

Absence of unequivocal dated evidence from elsewhere in South Wales has focused attention on coastal sections of Gower, where exposures of glacial and periglacial drifts overlying prominent raised beach fragments, have been regarded as key sites for establishing the Pleistocene chronology of the area[1,11]. Both Hoxnian[8] and Ipswichian ages have been suggested for these fragments; however, a composite origin within the Ipswichian has been established[7,14,16]. Bowen's limit for Devensian ice across Gower (Fig. 1*a*) rests largely on the acceptance that drifts containing erratics exposed in coastal sections east of Langland Bay and near Whitford Point are *in situ* Devensian glacial deposits, whereas exposures of erratic-bearing drifts associated with the local head west of Langland Bay along the south coast are regarded as redistributed Wolstonian glacial material[1]. A conjectural interglacial soil on Worm's Head[17] and soils considered to be in a colluviated state along the south coast west of Langland Bay[1] have also been cited as evidence in support of this ice limit. Other workers, however, have considered that Gower remained largely ice-free during the last glaciation[4,5] (Fig. 1*a*).

Recent coastal erosion at Broughton Bay (Fig. 1*b*) (SS: 419931) has revealed up to 5 m of a compact *in situ* diamicton containing broken and complete shells near its base (Fig. 1*c*). We interpret this sediment as a till; it is overlain by solifluction deposits and colluvium. The exposures run ~300 m eastwards from Twlc Point, where the till overlies fragmented raised beach sediments that contain a shell fauna comprising mainly *Littorina littoralis*. Fabric analysis (Fig. 1*b*) and erratic content of the till both point to a northerly (Welsh) provenance. Various marine shell species were removed from the basal 2 m of the till. The collection (Table 1) included 21 identifiable species, all of which live in the area now, except for *Arca lactea* and *Acanthocardia tuberculata*. These last two species are rare today and suggest slightly warmer conditions (N. McMillan, personal communication).

Table 1 Mollusca from Broughton Bay, South Wales

Gastropods	Bivalves
Patella vulgata	*Arca lactea*
Gibbula magus	*Mytilus edulis*
Littorina saxatilis	*Ostrea edulis*
Littorina littoralis	*Chlamys varia*
Turritella communis Risso	*Arctica islandica*
Nucella lapillus	*Acanthocardia tuberculata*
Ocenebra erinacea	*Cerastoderma edule*
Nassarius reticulatus	*Venus striatula*
Buccinum undatum	*Venerupis rhomboides*
	Venerupis pullastra
	Macoma balthica
	Corbula gibba

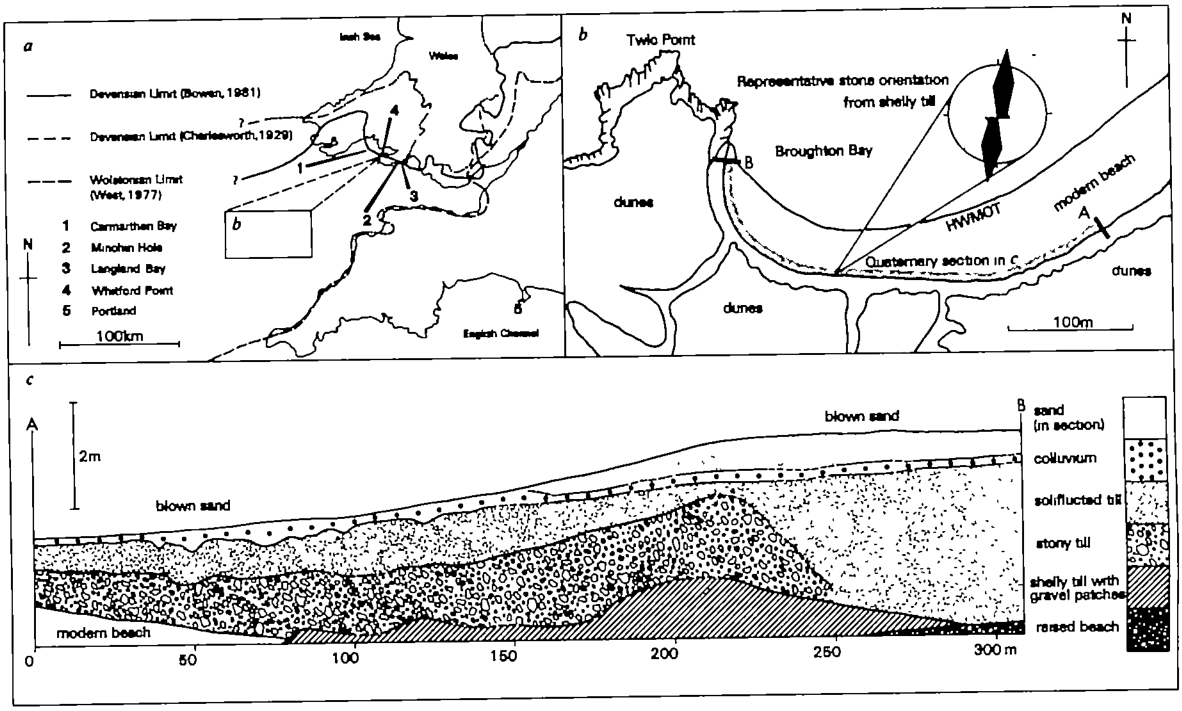

Fig. 1 South Wales. *a*, Site locations and suggested ice limits: ——, Devensian limit[7]; —·—·, Devensian limit[4]; – – –, Wolstonian limit[2]; *b*, site plan; *c*, generalized Quaternary stratigraphy, Broughton Bay.

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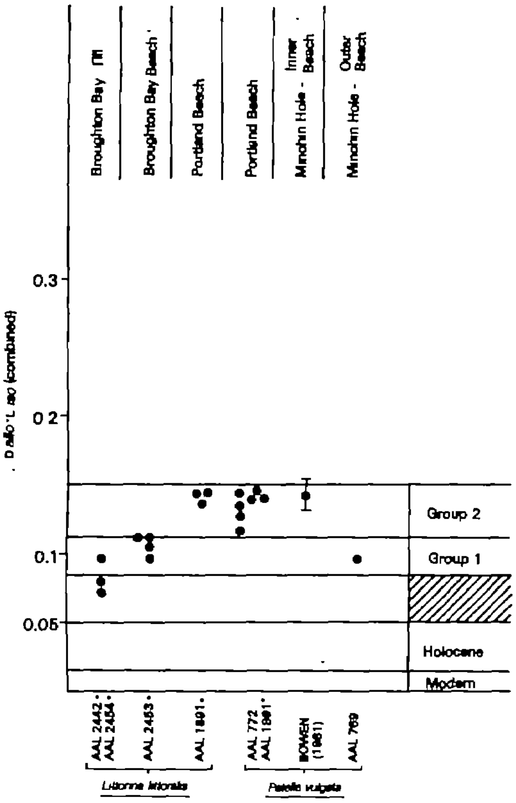

Fig. 2 Comparative amino acid ratios for south-west Britain (after ref. 14). * New data from the present results.

Littorina littoralis shells from the till and raised beach were prepared for amino acid analysis by the method described elsewhere[14,15], and the results are shown in Table 2. The tight clustering of the total ratios for these *Littorina* shells from the raised beach (AAL-2453) of 0.107±0.014 suggests strongly a non-mixed population. These values are lower than those obtained for *L. littoralis* from the raised beach at Portland (0.137±0.005) ($n = 3$, AAL-1891, A, B, C). (The ratios reported from Broughton Bay have been recalibrated against other samples run earlier. This procedure was necessary because detailed analysis involving many tens of runs indicated that the preparation method used for about 9 months in 1981 gave ratios that were consistently too high[18].) *Patella vulgata* from the same unit at Portland, collected by Briggs, gave total D *allo* : L *iso* ratios of 0.13±0.005 (AAL-1891, D, E, F). Comparable results derived from the 8 m beach on Jersey have been related to an absolute U-series date of $121\pm^{14}_{12}$kyr BP (ref. 13), and an average amino acid ratio of 0.14 for *P. vulgata* from the inner raised beach at Minchin Hole gives further indication of comparable relative ages for some of the raised beach fragments in south-west Britain[7]. The Broughton Bay ratios fall into group 1 of Andrews *et al.* (Fig. 2) and are thus probably younger than $121\pm^{14}_{12}$kyr BP.

The validity of these correlations rests principally on the acceptance of a comparable inter-site temperature history and of a similar kinetic relationship between the species *P. vulgata* and *L. littoralis*. The present mean annual temperatures of 10.9 °C at Gower (Swansea), 10.8 °C at Portland and 11.9 °C at Jersey (St Helier) are reasonably close. However, whether temperatures at these sites have consistently remained as close throughout the past 100 kyr, particularly during cold phases, is less certain. Taxon-dependent racemization differences are not judged to be a factor here on the basis of the close comparison between *L. littoralis* and *P. vulgata* (Fig. 2). Consequently, a correlation of the Broughton Bay raised beach with other beach fragments broadly regarded as Ipswichian (stage 5) in south-west Britain seems justified.

Table 2 Amino acid racemization results

Sample		Species	D *allo* : L *iso* (combined)	
AAL-2453	a	*Littorina littoralis*	0.115	Beach
	b	*Littorina littoralis*	0.107	
	c	*Littorina littoralis*	0.098	
	d	*Littorina littoralis*	0.115	
AAL-2442	a	*Littorina littoralis*	0.082	Till
	b	*Littorina littoralis*	0.073	
AAL-2454	a	*Littorina littoralis*	0.098	
Average D *allo* : L *iso* (combined):				
Littorina littoralis (Broughton Bay beach)			0.107±0.014	
Littorina littoralis (Broughton Bay till)			0.082±0.022	
Littorina littoralis (Portland beach)			0.137±0.005	
Patella vulgata (Portland beach)			0.130±0.005	

Littorina littoralis from the till yielded a slightly lower mean total D *allo* : L *iso* value of 0.082±0.022 than that from the raised beach (0.107±0.014) but still showed a relatively unmixed population. Hence, whereas the raised beach at Broughton Bay can be correlated by amino acid ratios with the outer beach at Minchin Hole, ratios for the till are on average slightly younger, although the ratios overlap at the 95% confidence level. A maximum relative age for the ice advance would be indicated by the ratio of the least racemized *L. littoralis* shell, namely 0.073±0.007.

The amino acid evidence therefore indicates that the shells in the till date from no earlier than the Ipswichian (~$121\pm^{14}_{12}$kyr BP (ref. 13)), and that some shells post-date the raised beach event at Broughton Bay. This evidence strongly suggests, therefore, that it must have been ice of Devensian age, and no older, which moving southwards across the eastern part of Carmarthen Bay, incorporated shell litter that had accumulated during the preceding interglacial. The fabric and erratic content of the shelly till deposited at Broughton Bay support this interpretation.

The point at which the Broughton Bay shelly till was deposited within the Devensian period cannot be resolved easily. The amino acid ratios themselves indicate the shell fauna is most probably early Devensian in age. This, however, only establishes a maximum age for a glacial event which incorporated the shells in the diamicton. On the other hand, stratigraphical[1,11] and palynological[19,20] data from the local area, point strongly to last glaciation deposits in the vicinity being late Devensian in age. The lack of amino acid ratios extending into middle or late Devensian times does not necessarily contradict the latter view; with sea level falling at the close of the Ipswichian, much of Carmarthen Bay would have remained as dry land during this period, preventing further accumulation of younger marine shells. Thus whilst the amino acid ratios presented above do not allow precise dating of the Broughton Bay shelly till within the Devensian period, they do demonstrate the encroachment of Devensian ice sheets on to west Gower. Furthermore, our results demonstrate the potential of amino acid ratios for resolving certain chronological aspects of the various shelly drifts of the UK and elsewhere[21].

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Comparison between the hot spot and geomagnetic field reference frames

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Hot spots are located on the Earth's surface and are caused by hot plumes of upwelling mantle material. They manifest themselves by volcanic activity which changes its location in response to the movement of the lithosphere over the mantle plume. It has been suggested[1–3] that hot spots are relatively stationary with respect to each other, and also that they represent a frame of reference with which to measure the absolute movement of the lithospheric plates over the surface of the Earth. Another frame of reference can be partially defined by the position of the mean magnetic dipole field of the Earth. As the dipolar field is believed to be closely related to the spin axis, this frame of reference automatically gives information about the palaeolatitude and orientation of the lithospheric plates. But since the position of the ancient dipole field of the Earth does not give any information about palaeolongitude, and since in many cases the definition of the field is relatively poor due to less than adequate rock collections during the time of interest, hot spot reference frames have been favoured by many people. We now report a comparison of the two frames of reference and show that over the past 200 Myr there has been relative movement between the two frames of reference of about 20°, and that this motion has not been constant with time.

We have developed a new method for calculating palaeomagnetic poles for individual continents[4]. As the relative positions of most continents are moderately well known during the Neozoic, it is also possible to use the palaeomagnetic information from one continent to define with greater accuracy the palaeomagnetic pole for another continent. Data for North America are shown in Table 1. The apparent polar wandering (APW) path shown on the left is that generated solely from North American palaeomagnetic data[4]. The APW path on the right has been generated using data from five continents (North America, South America, Africa, Europe and Australia) and using the relative rotation parameters given by Barron *et al.*[5] to express the palaeomagnetic poles of all continents in the North America frame of reference. The method of analysis of the rotated poles is similar to that described in ref. 4. There are two important results obtained by using the world data to define a North American polar wandering path.

First, the accuracy with which the pole may be defined can be significantly increased by using data from the other continents as well. The average value of α_{95} (the radius of the 95% circle of confidence around the mean) has decreased from 5.7° to 3.5° by using the data from Australia, South America, Europe and Africa in addition to those from North America, to define the North American APW path. Second, the two polar paths can differ by almost 10°, which occurs during the Upper Cretaceous and the Middle and Upper Jurassic. The ratio between the average values of α_{95} using just the North American data and using the data from all five continents is 0.61. If the relative positions of the continents were perfectly well known during the time interval considered, there should have been a reduction in the value of α_{95} equivalent to the square root of the ratio of information contained in the North American data to the information contained in the data from all five continents, which is 0.53. Thus, although the relative position information for the continents is not perfect, the reduction in α_{95} approaches that which would be expected considering the added information content and an assumption that the relative position information is correct. This is obviously not true in some cases; for instance the 200 Myr information shows no reduction in α_{95} whereas it should have been reduced from 2.8° to 1.6° if it were purely dependent on the amount of information used.

We compare the finite motions predicted by hot spots, and APW paths back to 200 Myr. Morgan[1] has defined the motions of the major continental plates with respect to a hot spot reference frame for this time interval. It is possible to use these data to generate pole positions on the assumption that the hot spot reference frame is fixed with respect to the geomagnetic field. We can then compare these calculated polar positions with those generated using palaeomagnetic data to see if the assumption holds true. We show three sets of results. The first set is given in Table 2 and compares the two polar wandering paths for Europe. The last column of this table shows the angular deviation between the two sets of results. Deviations increase from the present back to 50 Myr at which time there is a reduction in the deviation. From 70 to 180 Myr the deviation again increases and reaches a value of 17.6°.

When we generate polar wandering information for any continent we use relative position information which is different from that used by Morgan. Therefore, we obtain different results for different continents. This set of results (which

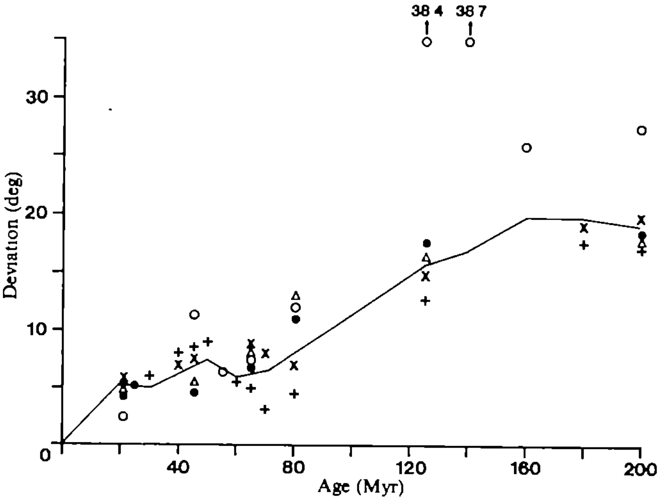

Fig. 1 Deviations between APW paths predicted using a hot spot reference frame and those using palaeomagnetic data. The symbols compare palaeomagnetic data in which relative positions between the continents used to combine the palaeomagnetic data are those given in ref. 5 with the hot spot data given in ref. 1. The line shows a comparison in which the relative positions of the continents used to combine the palaeomagnetic data are those given in ref. 1, compared with the same hot spot data. ○, Australia; +, Europe; △, Africa; ●, South America; ×, North America.

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Table 1 Apparent polar wandering path for North America

	Using North American data			Using data from five continents			
Age	Lat. (°N)	Long. (°E)	α_{95} (deg)	Lat. (°N)	Long. (°E)	α_{95} (deg)	Deviation (deg)
20	85.9	151.1	3.6	84.7	167.5	2.2	1.8
30	84.7	157.8	3.6	83.0	162.0	2.4	1.8
40	83.3	165.4	3.5	81.1	168.5	2.5	2.2
50	83.1	178.2	3.5	79.7	177.3	2.5	3.4
60	80.1	−176.2	4.7	78.2	178.7	3.0	2.1
70	76.7	−171.6	5.7	77.6	−178.3	3.6	1.7
80	70.6	−164.7	7.6	77.6	−176.6	4.3	7.7
90	68.8	−167.9	8.2	78.1	−179.4	4.1	9.8
100	69.1	−174.0	8.1	76.9	−174.6	3.7	7.8
110	69.5	−177.9	6.7	73.5	−168.0	3.5	5.1
120	67.9	179.1	6.3	71.9	−170.5	3.7	5.3
130	66.2	164.3	5.8	71.4	173.3	4.0	6.1
140	66.0	156.5	5.6	72.2	157.5	4.3	6.2
150	66.6	147.8	5.7	73.8	137.4	4.4	8.0
160	69.7	132.3	7.5	76.2	106.4	4.6	9.8
170	75.9	121.3	9.9	75.5	89.6	4.4	7.7
180	69.9	96.6	5.4	72.2	90.3	3.5	3.1
190	66.2	93.9	3.5	69.8	94.1	3.1	3.6
200	63.6	94.1	2.8	68.4	94.7	2.8	4.8
Average			5.7			3.5	

includes the values for the last column in Table 2) is presented in Fig. 1, in which the scatter for any one age reflects the fact that our relative position information is different from that given by Morgan. In particular, the very large deviations seen for Australia between 125 and 140 Myr are due to the fact that Morgan's position for Australia during these times is such that the APW path for Australia would predict poles very close to the present pole, whereas in our reconstruction Australia lies considerably further south and is rotated compared with its present position. It should be possible to use palaeomagnetic data to determine which of these reconstructions is better, but unfortunately, there are almost no data for Australia during this time interval[6]. The points plotted in Fig. 1 suggest an increase in the deviation between the present and about 40 Myr before which there is a suggestion of a slight decrease in the deviation. The deviation increases between about 70 and 180 Myr. The close grouping of the data from the four continents surrounding the Atlantic Ocean at 200 Myr is because our reconstruction of Pangaea[7] is very similar to that of Morgan[1], the only major difference relevant to this study being in the relative position of Australia.

Duncan[8] has also produced absolute motion parameters of the southern continents with respect to a hot spot frame of reference, back to 100 Myr ago. Deviations calculated using his data fall within the scatter of the points shown in Fig. 1.

Table 2 Comparison between observed APW path for Europe and that predicted by motion of Europe w.r.t. the hot spots

	Predicted APW position		Observed APW position*		
Age	Lat. (°N)	Long. (°E)	Lat. (°N)	Long. (°E)	Deviation (deg)
21	87.7	61.2	85.1	150.3	5.4
30	86.7	79.8	83.5	144.3	5.9
40	85.6	77.8	82.0	150.5	7.9
45	84.8	79.8	81.7	153.4	8.5
50	84.4	81.5	81.3	156.3	9.0
60	84.3	134.1	80.5	165.6	5.5
65	83.6	143.7	80.5	172.3	4.9
70	81.0	159.9	80.5	178.9	3.1
80	75.9	184.7	80.3	189.8	4.5
125	67.3	170.9	73.6	204.3	12.6
180	52.7	116.6	70.2	113.5	17.6
200	49.5	110.4	66.6	118.6	17.6

* Observed APW position calculated using data from all five continents plus the relative position information.

We have also generated another global polar wandering path using the same palaeomagnetic data but using the relative position information given by Morgan[1]. The polar wandering path for North America is in fact very similar to that given in Table 1. Deviations between the two average 2.5°, the largest being 6.2° at a time of 160 Myr. The inaccuracy for each pole is marginally greater for Morgan's reconstruction data (mean $\alpha_{95} = 3.6°$). Comparison between this polar path and the hot spot data need be done only once, since the same relative position information was used for both sets of data. The deviation between the two is shown by the line in Fig. 1.

The average motion between the two frames of reference during the 200 Myr interval of observations is about 19°, representing a speed of motion of 10.6 mm yr^{-1}. The relative speed of motion was faster during the period 60–160 Myr, being about 15.6 mm yr^{-1}. The motion established by the point at 21 Myr gives a speed of motion between the two reference frames of 28.0 mm yr^{-1}, but the error in this number is much greater because it is based on a smaller deviation.

A word must be said about errors, Presumably, the hot spot traces, being marked by geological features, are accurately placed to within 1°. There may, however, be errors associated with assigning ages to the traces. Because most of the hot spots used by Morgan are within continental regions at the start of his reconstruction, the ages should be much better known than for oceanic hot spot traces. A typical radiometric age has an error of 3% of the total age, or 6 Myr in the case of the 200 Myr hot spot traces. At a motion of 30 km Myr^{-1}, this age error is equivalent to an angular error of about 1.6°. Thus a possible hot spot error might be given by the square root of the sum of the squares of 1.6 and 1, or 1.9°. The polar error could likewise be considered to be made up of errors due to the palaeomagnetism (average of 3.5°, Table 1) and errors due to plate reconstruction (taken as the mean polar difference between Morgan's relative position data and ours of 2.5°) giving a total palaeomagnetic polar error of 4.3°. The total possible error in the deviation will be in the region of 4.7°, and so the observed deviation of over 19° observed at 200 Myr represents a real effect. Possible errors in the reconstruction of Gondwanaland will have little effect on the overall result since only 7% of the palaeomagnetic data come from Australia. The remaining 93% of the data come from Europe, North and South America, and Africa, whose relative positions are well known apart from minor differences between various models.

Morgan[1] also showed that there was relative motion between his hot spot reference frame and the geomagnetic field. But the greater number of palaeomagnetic data that we have used has described the effect more accurately. The details of the relative motion revealed by this study also differ from those suggested by Morgan. This relative motion between the hot spot and the geomagnetic field reference frames is considerably greater than the relative motions which take place between the hot spots which are about 5° in 200 Myr according to Morgan. If this supposition is correct, it is necessary for the layer within the mantle at which the hot spots are generated to be drifting slowly with respect to the spin axis of the Earth, to which we assume the geomagnetic field is fixed.

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Thermal convection in magmas generated by hot-plate heating

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A condition is derived here for the onset of thermal convection in a layer of silicate partial melt that is generated by suddenly and continuously supplying an anomalous heat flux or by maintaining an anomalous high temperature on a plane at some depth within the Earth so that the overlying material is heated to greater-than-solidus temperatures. The results show that for highly fluid basaltic magmas convection may begin in a layer only 10-m thick, whereas for viscous rhyolitic magmas, at least a 10-km thick layer is required. The fact that thermal convection may occur within such a thin layer of basaltic partial melt may partially explain the homogeneity of large volumes of flood basalts that occur. Since convection within rhyolitic partial melts is unlikely, homogeneous rhyolites must be the result of mixing in magma chambers.

Although the hot-plate model is an oversimplification of the process of melt generation within the Earth, it may be relevant to the generation of rhyolite magmas near subduction zones, where mafic intrusions may supply heat to melt partially the overlying crust. Hodge[1] has suggested such a model for the generation of granitic batholiths. Hodge's calculations show, for example, that the heat transferred from a cooling mafic sill 4 km thick, initially at 1,200 °C, that is emplaced in country rock initially at 500 °C and which has a melting temperature of 700 °C, is sufficient to generate nearly a 1-km thick layer of partial melt of granitic composition within 40,000 yr. Hodge did not discuss whether convection could occur within the partial melt layer, however.

The nature of the heat source that would act as a hot-plate for the generation of basaltic magma in the mantle cannot be specified with certainty. Yoder[2] has suggested that a peridotite diapir that is depleted of its basaltic constituents or that an inflection in the geotherm might provide the required heat flux.

Although the hot-plate model is an approximation to natural mechanisms of magma generation, the model is a useful first step towards an understanding of magma genesis and transport. Moreover, the hot-plate process is amenable to laboratory investigation. Yoder[3] is carrying out experiments on the onset of convection in a two-component silicate partial melt heated from below. The present calculation may provide a useful tie between theory and experiment.

A simplified schematic of the hot-plate model is shown in Fig. 1. In reality the partial melt region is very complicated. In a multi-component system, the partial melt fraction and its compositions are a function of temperature, pressure, and bulk composition of the source rocks; consequently, the physical parameters of the system will vary as a function of height in the partial melt layer. It is not possible to take into account changes in density, specific heat, thermal conductivity, viscosity, permeability, and so on; so the conditions for the onset of thermal convection in a growing layer of silicate partial melt will be determined by assuming a system with uniform properties and by assuming melting at a fixed temperature.

Observations on the partial melting of silicate rocks in laboratory experiments have shown that when partial melts form, the liquid coats the grain boundaries of the remaining solid matrix[4,5]. The melt thus provides porosity and permeability within the partial melt, and the magma may flow along grain boundaries. The dynamics of the magma will be assumed to be governed by Darcy's law for flow in a porous medium[6–9].

The problem is to determine the condition for the onset of convection in a fluid-saturated porous medium heated from below, where the height of the layer grows with time in a manner determined by the rate of advance of solidus boundary. When the layer reaches a certain critical thickness, the conduction regime will become unstable and thermal convection will be set up. The condition for the onset of convection is expressed by a dimensionless parameter termed the Rayleigh number, the exact form of which depends on the boundary conditions that are applied[10]. The bounding surfaces are assumed here to be rigid and the temperature at the top is the melting temperature T_m, and at the base of the layer a fixed temperature T_1 will be maintained. With these boundary conditions, the critical Rayleigh number, R, is $4\pi^2$ (ref. 10). Thus:

$$R = \frac{\rho_t s \alpha g X(t)^2 K \langle \partial T_0/\partial x \rangle}{\lambda \nu} \geq 4\pi^2 \qquad (1)$$

where ρ_t, s, and α are the density, specific heat, and thermal expansion coefficient of the melt, respectively; g is the acceleration due to gravity; $X(t)$ the thickness of the partial melt layer; K the permeability; $\langle \partial T_0/\partial x \rangle$ the mean conductive temperature gradient maintained across the layer, λ the thermal conductivity of the solid–fluid mixture, and ν the kinematic viscosity of the melt.

The mean conductive gradient is found from a solution to the Stefan problem[11]:

$$T_0(x, t) = T_m + \Delta T\{1 - [\operatorname{erf} x/2(at)^{1/2}]/\operatorname{erf} Q\} \qquad (2)$$

where $\Delta T = T_1 - T_m$, $a = \rho_t s/\lambda$, and Q is found from the relationship

$$Q\,(\operatorname{erf} Q) \exp(Q^2) = St/\sqrt{\pi} \qquad (3)$$

where the Stefan number, St, is defined as $St = s\Delta T/\chi L$; χ is the partial melt fraction and L is latent heat of melting. The motion of the phase boundary is given by:

$$X(t) = 2Q(at)^{1/2} \qquad (4)$$

From equation (2), the mean temperature gradient is given by $\Delta T/X(t)$.

The interconnected pathways that provide the permeability within the partial melt zone are undoubtedly quite complex. Nevertheless, a reasonable approximation to the permeability can be obtained by assuming that the solid matrix is cubical with average grain diameter b, and that the melt fills the interconnected channels of width d. It is assumed that there are no isolated pockets of partial melt. The porosity, which can then be defined as the degree of partial melt, χ, is given by[8] $\chi = 3\pi d^2/4b^2$ and the permeability by[12] $K = \chi d^2/96$. Eliminating d gives $K = \chi^2 b^2/72\pi$. On substituting for K and $\langle \partial T_0/\partial x \rangle$ into equation (1) gives the condition:

$$X(t) \geq \frac{288\pi^3 a \nu s}{\alpha g \chi^3 b^2 L St} \qquad (5)$$

Yoder[3] has suggested that 30% partial melt of basaltic composition is generated at the invariant point in the eutectic-like

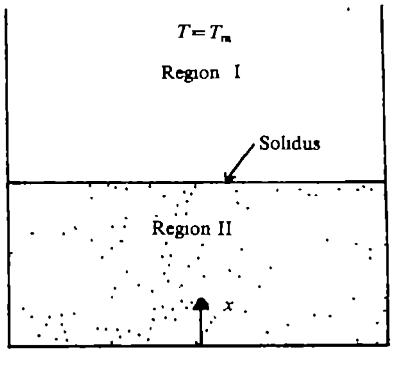

Fig. 1 Schematic of hot-plate heating model. Region I is solid at the melting temperature T_m. Region II is partially molten. A fixed temperature $T_1 > T_m$ is maintained at $x = 0$.

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Table 1 Values of parameters considered

Parameter	Value	Comments
ν	$10-10^6\ \mathrm{cm^2\ s^{-1}}$	See text.
a	$10^{-2}\ \mathrm{cm^2\ s^{-1}}$	Typical igneous rock values.
s	$0.25\ \mathrm{cal\ g^{-1}\ °C^{-1}}$	
α	$3\times10^{-5}\ °\mathrm{C}$	
g	$10^3\ \mathrm{cm\ s^{-2}}$	
χ	0.3	See text.
b	10^{-1}–1 cm	
L	$135\ \mathrm{cal\ g^{-1}}$	See text.
St	0.2–2.0	Corresponds to a temperature range of 32–320 °C across the partial melt layer.

melting of garnet-peridotite and that the degree of partial melt increases with temperature. The degree of melt for rhyolites depends on the bulk composition, among other factors. Some rhyolite compositions, minimum melt rhyolites, form nearly 100% melt at a fixed temperature[3]. A conservative average value of $\chi = 0.3$ is assumed. The assumption of a fixed melting temperature T_m in this case means that 30% of the material melts at T_m; the rest of the material remains solid until T is considerably greater than T_1.

The latent heat of fusion is taken to be 135 cal g^{-1}, the value given by Yoder[13] for garnet-peridotite. The latent heat for rhyolite may be lower. Table 1 lists the numerical values of all the parameters used to evaluate equation (5). Substitution of the values gives

$$X(t) = 2\times10^2\ \nu/b^2St \qquad (6)$$

where ν, b are given in c.g.s. units.

Figure 2 gives a plot of equation (6) as a function of ν for a few representative values of b and St. The results show that for low viscosity, which is characteristic of basaltic magmas[14], and coarse-grained source rocks, that convection will begin in a layer of partial melt as little as 10-m thick. With smaller values of the grain size or Stefan number, or larger values of viscosity, convection will set up in a layer of 1-km thickness or less. From equations (3) and (4), the time required to generate a 1-km thick layer, when $St = 2$, is $\sim10^4$ yr, which is not an unreasonable time for a thermal event. From these calculations it would seem that convection will probably occur in the partial melt zone, and thus homogenize the magma before its leaving the source zone. This effect may partially explain the large volumes of nearly homogeneous basaltic magma that have been episodically extruded throughout geological history.

On the other hand, if the viscosity is of the order of 10^4–10^6 cm^2 s^{-1}, as may be expected for rhyolitic magmas[15], and the other parameters are held fixed as in Table 1, the thickness of the partial melt layer must exceed 10 km before convection will begin, even for the most ideal case shown in Fig. 2. For this case, the time required for generation of the layer must exceed 10^6 yr. These values of layer thickness and time required for convection to begin in a rhyolitic partial melt are not totally out of line, but they must be viewed as lower bounds. If, for example, $b = 10^{-1}$ cm or $\nu = 10^6$ cm^2 s^{-1} and the other values are held constant, $X(t) = 10^3$ km. This thickness is clearly unrealistic. It is not likely that modifications in the other parameters will alter this result much. In particular, for the porous medium model to hold χ must be ≤ 0.5. For a greater value than this, the solid matrix structure will collapse[16]. Moreover, Ahern and Turcotte[8] have shown that the matrix will begin to collapse at the bottom of the partial melt layer as a result of differential stresses, when the thickness of the layer is of the order of 100 m. Finally, if a rhyolitic partial melt layer extends to fairly shallow depths, brittle fracture may occur above the layer and the magma may escape before being mixed by convection within the source zone. Thus if rhyolitic magmas are homogenized at any time, the convection process must occur in subsidiary magma chambers.

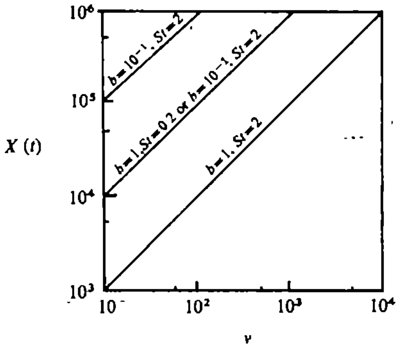

Fig. 2 Thickness $X(t)$ of partially melt layer at initiation of thermal convection as a function of viscosity ν for typical values of grain size b and Stefan number St. All dimensions are c.g.s.

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Pattern of opening rates along the axis of the Mid-Atlantic Ridge

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Vine and Wilson[1], using the Vine and Matthews[2] hypothesis, were the first to try to relate the magnetic pattern over the crests of the mid-ocean ridges to the known geomagnetic time scale to determine the spreading rate over the past few million years. Many determinations of spreading rate at the axis of the ridge during Plio-Pleistocene times have been published: Le Pichon[3] and Morgan[4], for example, used spreading rates computed for the axial magnetic pattern (10 Myr: anomaly 5 of Heirtzler *et al.*[5] or for the past 5 Myr respectively) even when trying to assess the properties of instantaneous plate kinematics. Likewise in the systematic inversion of Minster *et al.*[6] anomalies 3 and 5 were generally used thus averaging plate speeds over the past 5–10 Myr. Minster and Jordan[7] sought to improve this and redetermined the spreading rates using magnetic anomalies 2 and 2′ yielding a mean averaging interval of <3 Myr. Here we examine plate behaviour over a shorter time scale in the reasonably well-surveyed axial region of the Mid-Atlantic Ridge south of the Azores. The average opening rates we obtained for this region for magnetic anomalies 1 (Brunhes), 2 and 2′ using two different geomagnetic time scales indicate that (1) there was a relative slowing down of the opening between anomalies 1 and 2 which may be of global significance; (2) the opening is compatible with a rigid plate model when it is averaged over ~2.5 Myr although for shorter averaging times (1.8 and 0.7 Myr) there is some evidence of non-rigidity of the plates. Thus different segments of the Mid-Atlantic Ridge may display an independent behaviour and, with respect to relatively short time intervals, may show departures from characteristics typically associated with slow accretion.

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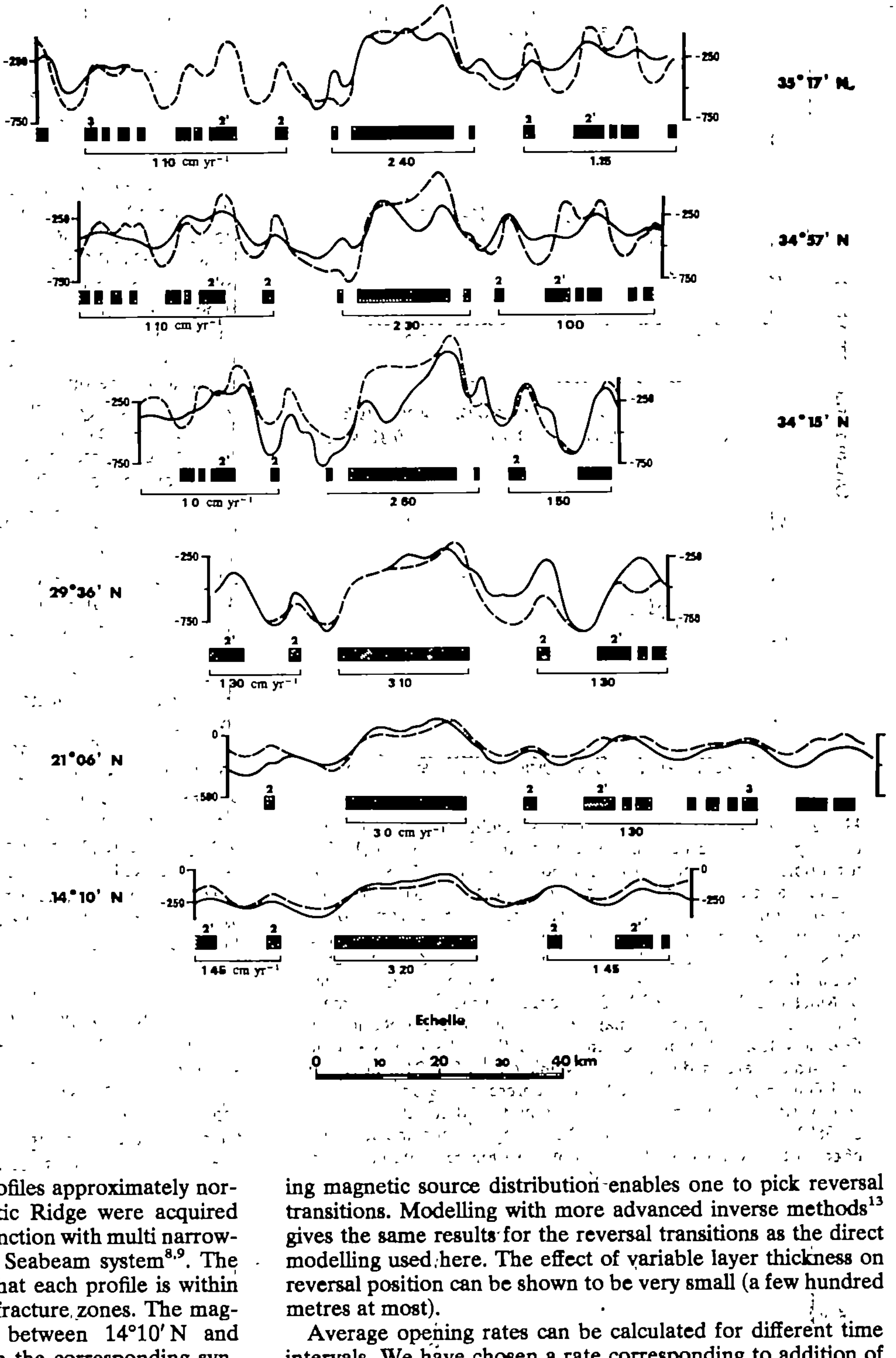

Fig. 1 Magnetic anomaly profiles along R/V *Jean Charcot* tracks during the *Vema* cruise, (solid lines) at six locations along the Mid-Atlantic Ridge south of the Azores. IGRF 75[26] has been removed from the total intensity data. The synthetic anomaly profiles (dotted lines) have been constructed piecemeal from the distribution of a uniformly magnetized horizontal magnetic source layer shown in hatched (positive polarity) and white (negative) below each profile pair. The depth to the top of the source layer is, from north to south, 2,000–2,300–2,800–3,100 and 2,500 m. This corresponds to the average ocean depth along each profile. The azimuth of the observed magnetic profiles is, from north to south, 94–101–100–107–104 and 90°. The profiles are approximately normal to the local strike of the ridge. Modelling includes the Jaramillo event for the three northern profiles. For the other profiles the axial rate applies only to the Brunhes block and Jaramillo is not resolved.

Six R/V *Jean Charcot* magnetic profiles approximately normal to the strike of the Mid-Atlantic Ridge were acquired during the 1977 *Vema* cruise in conjunction with multi narrow-beam bathymetry obtained with the Seabeam system[8,9]. The Seabeam bathymetry demonstrates that each profile is within one given crustal block bounded by fracture zones. The magnetic anomaly profiles distributed between 14°10′ N and 35°17′ N are presented in Fig. 1 with the corresponding synthetic profiles computed from the magnetic source distribution shown below each profile pair. The magnetic modelling used to compute synthetic profiles is quite simple: in each case we have assumed an horizontal magnetic source layer of uniform thickness (400 m) and intensity (0.0024 e.m.u. for all profiles except for the profile at 34°57′ N where the magnetization has been chosen as 0.02 e.m.u.). We also assume accretion to take place on a line of zero width. The uniform depth of each source layer has been chosen to match the average ocean depth along each Jean Charcot profile to take into account the varying distance to the magnetic source layer due to topographic variations along the strike of the Mid-Atlantic Ridge[10,11].

Synthetic anomaly profiles were generated at several rates using the Talwani *et al.*[12] time scale and the match between observed and synthetic anomalies was done piecemeal. Thus the synthetic profiles shown in Fig. 1 are composite. The resulting magnetic source distribution enables one to pick reversal transitions. Modelling with more advanced inverse methods[13] gives the same results for the reversal transitions as the direct modelling used here. The effect of variable layer thickness on reversal position can be shown to be very small (a few hundred metres at most).

Average opening rates can be calculated for different time intervals. We have chosen a rate corresponding to addition of new crust during the Brunhes epoch (anomaly 1; 0.69 Myr) and the average rates since the times (1.78 and 2.635 Myr) corresponding to the middle of anomalies 2 and 2′ respectively according to the time scale of Talwani *et al.*[12]. The observed opening rates averaged over these three different time intervals are plotted as a function of latitude in Fig. 2*a* which also shows the data obtained in the Famous area around 36°58′ N by Greenewalt and Taylor[14], Needham and Francheteau[15], Renard[16] and MacDonald[17]. The opening rate data are compared with the pattern of opening predicted by the inversion model of Minster and Jordan[7] for the Mid-Atlantic Ridge south of the Azores using bounds derived from the inversion model uncertainties. We compare opening rates computed along the ship's profiles which are nearly normal to the local strike of the ridge crest on the basis of Seabeam data with opening rates calculated along the direction of relative motion of the plates.

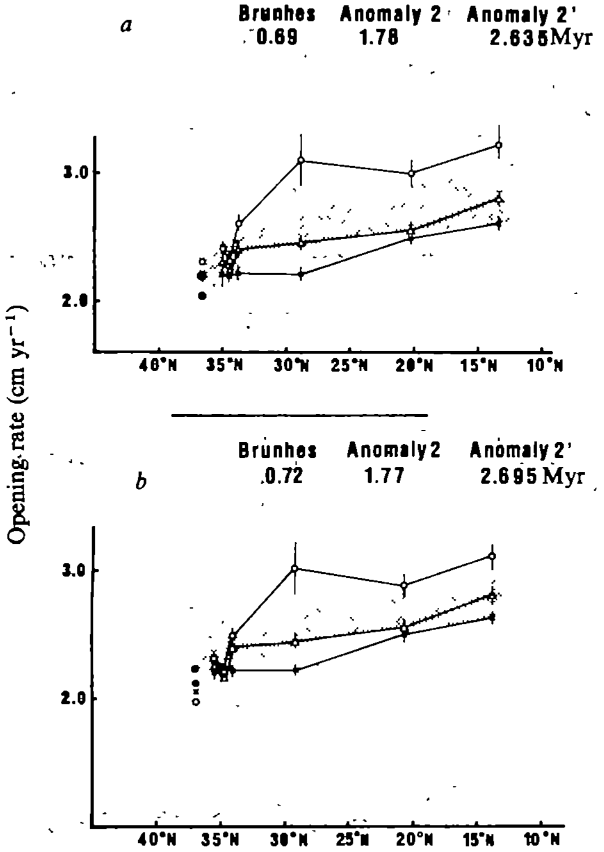

Fig. 2 Along-strike distribution of spreading rates. The total opening rates have been calculated for the Brunhes epoch (O), for a time span extending to the middle of anomaly 2 (×) and for a time span extending to the middle of anomaly 2' (△). Error bars for spreading rates have been estimated from a comparison between the synthetic model and the observed profiles. Three rates have been estimated for the latitude of the FAMOUS area at 36°58' N: a rate of 2.04 calculated for anomaly 1 and 2 times by MacDonald[17]; a rate of 2.2 for anomaly 1 time[15], up to anomaly 2 (ref. 27) and up to anomaly 3[16]; a rate of 2.3 obtained by Greenewalt and Taylor[14] for anomaly 1 time. Two geomagnetic time scales were used (Fig. 2*a*, Talwani *et al.*[12]; Fig. 2*b*, Mankinen and Dalrymple[18]. The shaded band represents the opening rates computed from the pole of Minster and Jordan[7] at 80.43° N–56.36° W (angular rotation rate 0.258° Myr^{-1}) and includes the effect of the oval of confidence in pole position computed by these authors.

The difference between the two directions is always <10° and thus the correction factor never exceeds 0.984. The pattern of observed rates falls into the permissible band only when the averaging is conducted over a time interval of ~2.6 Myr. This is also true, although less so, when the data are averaged over 1.78 Myr. It is apparent from Fig. 2*a* that there is a departure from Minster and Jordan's model or any other rigid plate model when observed opening rates are averaged over the relatively short Brunhes time interval. The profile that yields the largest departure, at 29°36' N, corresponds to a misfit of 3.7 km in the position of the Brunhes reversal. This cannot be accounted for by the uncertainty in the model. To test the influence of the time scale which we have used we recomputed the opening rates (Fig. 2*b*) with the time scale of Mankinen and Dalrymple[18]. Although the discrepancy between observed and model rates is reduced, the data are clearly not compatible with rigid plate behaviour. In particular the opening rate observed at 29°36' N for the Brunhes epoch is greater than that observed at 21°06' N for the same time interval irrespective of the time scales. There is also evidence, in the region around 35° N where several data points are closely spaced, for strong departures from rigid plate behaviour even for a 2.6 Myr averaging interval. We will not emphasize this result, however, in the absence of a detailed survey, because the differences in opening rates are of the same order as the uncertainties in the determination of rates. The fact that, when averaging is done over about 2.6 Myr, the observed rates fall in the slower portion of Minster and Jordan's band may be evidence that the angular velocity for the North America–Africa motion computed by Minster and Jordan is too high.

We note in Fig. 2 the differences in opening rate north and south of 30° N. One may be tempted to locate a plate boundary at this latitude but it finds no support in the geology. We also note in Fig. 2 that the opening rates during the Brunhes epoch are systematically greater than the rates averaged over longer time intervals. To examine in more detail the opening rate variations as a function of time, we present in Fig. 3 the spreading rates for the east and west limbs of the ridge for the Brunhes epoch (0–0.69 Myr BP), the latter part of the Matuyama epoch (0.69–1.78 Myr BP) and the period before the Gilsa event (before 1.87 Myr). To compute a spreading rate for the Brunhes epoch we assume that the location of maximum depth of the rift valley within the inner floor[15] is the locus of accretion coinciding with zero age. Bathymetric information from Seabeam is very convenient for this purpose. The spreading rates for the other two time periods are derived directly from the models in Fig. 1. Two observations are apparent. First the distribution of spreading rates is approximately symmetric

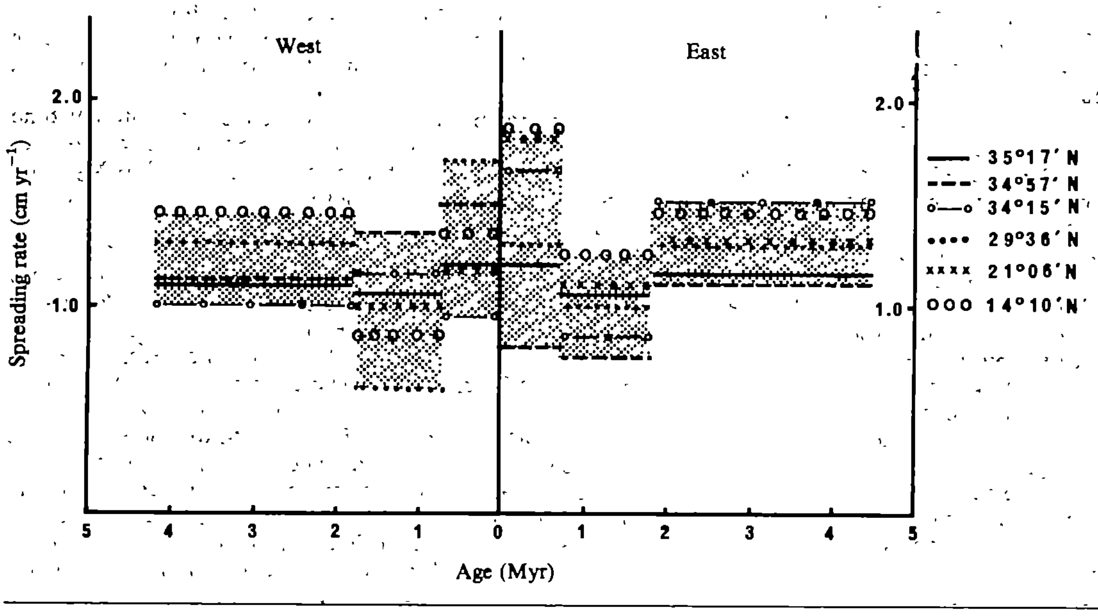

Fig. 3 Variation of spreading rates as a function of time. The spreading rates are keyed to their respective profiles. They are calculated for three time intervals: 0–0.69 Myr (Brunhes; anomaly 1), 0.69–1.78 Myr (Brunhes–Matuyama boundary to middle of anomaly 2), before 1.78 Myr.

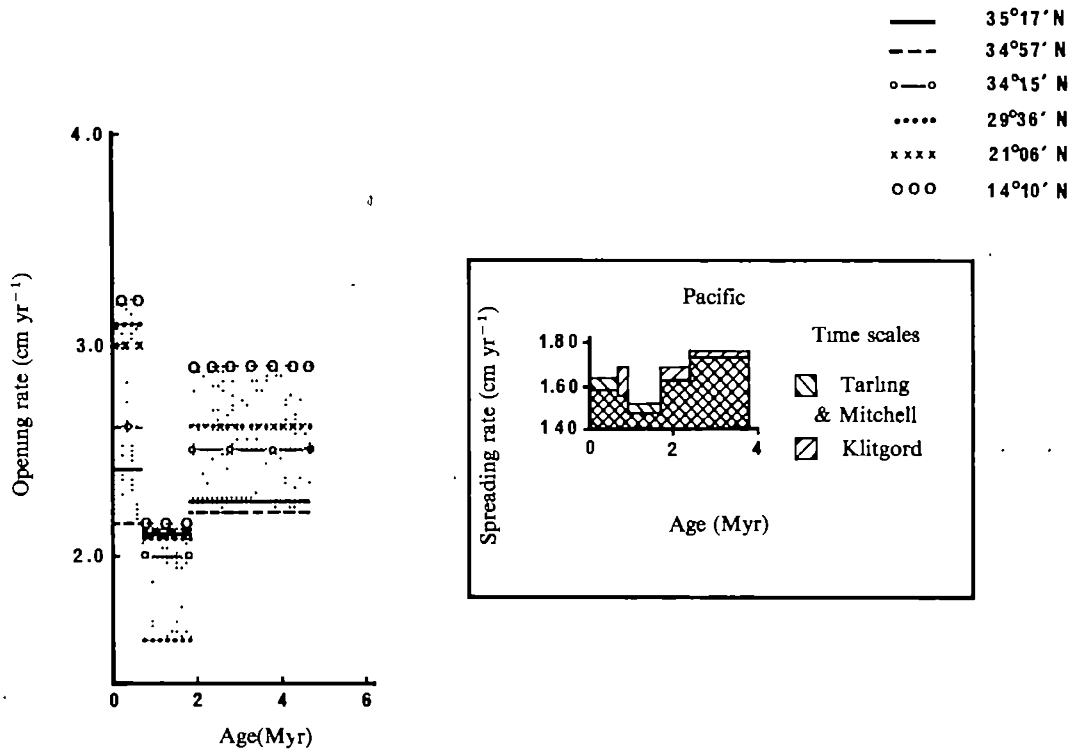

Fig. 4 Variation of opening rates as a function of time (derived from Fig. 3). Inset: variation of opening rates along the East Pacific Rise and Juan de Fuca Ridge (after Rea and Scheidegger[19]).

about the ridge axis—no one side is accreting crust systematically faster than the other side. Second, the Brunhes epoch is characterized by a distinctly faster rate of spreading compared with time intervals up to 4.6 Myr. Similarly the latter part of the Matuyama epoch from 0.69 to 1.78 Myr BP is characterized by smaller spreading rates.

One can ignore the distinction between east and west accretion because of the approximate symmetry about zero age in Fig. 3 and examine the variation of total opening rates as a function of crustal age (Fig. 4). There is a definite decrease in accretion rates for the 0.69–1.78 Myr BP period. The decrease is correlated with a change in spreading direction (H. Schouten, personal communication) particularly near the Kane fracture zone at 24° N. Rea and Scheidegger[19] also observed a definite slowing-down in plate accretion at the axis of the Juan de Fuca ridge (Fig. 4) which they linked to intraplate volcanism (Hawaii) and the dynamics of subduction[19]. We bring here some evidence that the spreading rate variations may be of global significance.

When accretion of the Mid-Atlantic Ridge is examined over a short time interval (<2 Myr) there is evidence that the oceanic plates do not behave rigidly. If we average the opening over a longer time interval (~2.6 Myr) the pattern of opening along the accreting plate boundary fits with that predicted by rigid plate behaviour. The evidence that we presented is derived from a rather simple modelling of magnetic anomalies but it is corroborated by more advanced modelling techniques[13]. The results are very sensitive to the position of the magnetic reversals but are not dependent on the number of sample points used in the 14–35° N interval. Thus, although we have used only a very limited data set, the departure from a rigid plate model is considered significant if one accepts the model results of Fig. 1. The modelling is strengthened by the fact that we have used magnetic profiles on two-dimensional bathymetry. The non-rigid opening between plates over short time intervals is not surprising and is consistent with the episodicity at the axis of mid-ocean ridges required by volcanic and tectonic data[20–22], geochronology[23,24], geochemistry and petrology (C. J. Allegre, personal communication) and hydrothermal activity (ref. 25 and R. D. Ballard, personal communication). It is clear that different segments of the Mid-Atlantic Ridge display an independent behaviour and, with respect to relatively short time intervals, may show departure from characteristics typically associated with slow accretion. This may provide a mechanism for initiating small transform faults. The size of each independent segment is not known, however, nor is known the behaviour of the ridge accretionary process over shorter time intervals for want of additional magnetic markers in the Brunhes epoch. One will probably need high resolution biostratigraphy for this purpose.

There is also evidence that the fluctuations of spreading rate with time may be global in nature although a close look is needed at other parts of the Atlantic and Pacific oceans and also in the Indian Ocean.

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Ultrathin mycelia-forming organisms from submarine volcanic areas having an optimum growth temperature of 105 °C

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The most extremely thermophilic organisms known to date have been isolated from continental volcanic areas[1–3], and grow optimally between 70 and 85 °C. In the hope of finding organisms living at temperatures above 100 °C I have taken samples from the hot sea floor of a submarine solfatara field where, as a result of the high pressures liquid water is found that is hotter than 100 °C. Here I report that, from these samples, I isolated unusual disk-shaped prokaryotic organisms, connected by a network of thin hyphae, which grew at 100 °C in the presence of sulphur, hydrogen and carbon dioxide. The organisms could be successfully transferred to synthetic media and were strict anaerobes, growing optimally at 105 °C and not at all at 80 °C or below. However, they did survive for long periods at 4 °C and −20 °C in the absence of oxygen. During growth, H_2S was formed by sulphur reduction. Due to their extreme oxygen sensitivity, their unusual, irregular shape and primitive volcano-adapted metabolism, the novel organisms may represent a very ancient form of life existing in submarine volcanic areas in a water-supplied oxygen-free habitat, which has not changed for billions of years and which prevents competition with normal life due to prohibitively hot temperatures.

Extremely thermophilic organisms from submarine volcanic habitats have not been isolated previously. It has been assumed[4] that due to their high salinity, hot marine waters may contain less caldoactive organisms than the hot fresh waters occurring on the continent. Samples were collected from a submarine solfataric field close to the bay of Porto Levante, Vulcano Island, Italy. The sea floor was situated at a depth between 2 and 10 m and consisted mainly of sandy sediments with many small craters, and rock formations with gas-spewing, sulphur-encrusted holes and cracks. Temperatures of up to 103 °C were measured in the sediments and in the cracks and holes. Rubber gloves were used during sample collection to provide protection from the heat. The sediment was stirred as heavily and deeply as possible and material was then drawn into a syringe fitted with an enlarged inlet. The samples, still hot, were taken to the surface and transferred to storage bottles, which were immediately sealed with rubber stoppers, and then traces of oxygen were removed by injecting sodium dithionite[5]. Microscopic inspection of the samples revealed a small number of unusually flat organisms.

In the laboratory, sterile seawater was inoculated with the samples (5% inoculation) and incubated at 100 °C in the presence of sulphur and an H_2/CO_2 atmosphere (80:20 v/v; 2 bar pressure) with strict exclusion of oxygen[6]. Of the 21 samples taken from the sandy sediments and cracks, four yielded disk-shaped organisms which grew within 2 days. The original temperatures of the samples were all ~100 °C. The enrichment cultures were transferred successfully to a medium consisting of synthetic seawater supplemented with trace minerals[6], KH_2PO_4 (0.5 g l^{-1}), and sulphur (15 g l^{-1}). Growth occurred only in the presence of H_2 and CO_2. H_2S, which was formed as an end product, was identified as cadmium sulphide. During growth, the sulphur flowers became greyish and appeared corroded. After 1 week, the sulphur supply was depleted. Uninoculated controls neither showed this phenomenon nor produced H_2S. These observations indicate that the novel organisms acquire their energy by a sulphur–hydrogen autotrophy similar to that recently described for a rod-shaped bacterium from Iceland (ref. 7 and F. Fischer, W. Zillig and K. O. Stetter, in preparation).

The enrichment cultures were purified by serial dilution. During exponential growth (Fig. 1*a*,*b*), the pure cultures exhibited a doubling time of 550 min at 85 °C and of only 220 min at 100 °C (Fig. 1*b*). Surprisingly, the isolates grew optimally at 105 °C with a doubling time of 110 min (Fig. 1*b*). The isolates were transferred (1% inoculation) to fresh medium five times in succession and incubated at 105 °C. Microscopic inspection showed about the same high cell density in the last tube as in the first, indicating that the organisms multiplied at this temperature. At 110 °C, very weak growth occurred (not shown). It remains unclear whether this represents the upper temperature boundary, or whether, more probably, the reduction of growth was due to the sintering of the sulphur forming a lump and therefore rendering it inaccessible to the organisms. Below 80 °C, the isolates were unable to grow (not shown). However, they could be re-isolated from the original anaerobic samples, stored at 4 °C and at −20 °C over a period of at least 10 months. In the presence of oxygen, however, they were inactivated within hours.

Under the light microscope, the organisms appeared as flat disks, highly variable in diameter (from 0.3 to 2.5 μm), some having an indented, dish-like appearance. They were usually arranged in single file or in other formations with constant distances between each other. Under the electron microscope, most of the disks appeared to be connected by ultrathin rods up to 40 μm long (which are possibly hyphae; Fig. 2*a*) and only

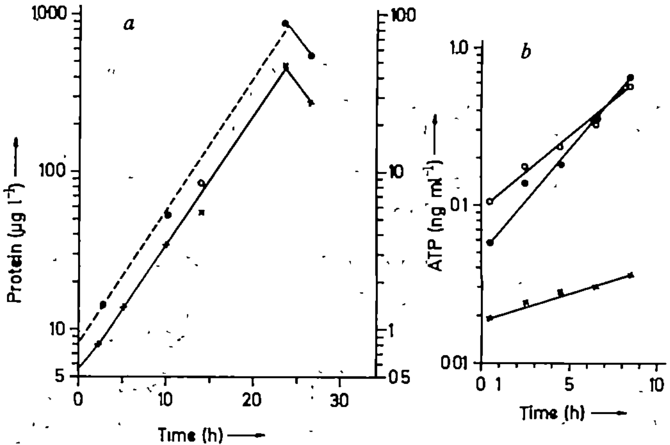

Fig. 1 Growth curves of the isolate PL-19. *a*, Growth in a 10-l fermenter at 95 °C: (×) ATP concentration; (•) protein concentration. The fermenter contained artificial seawater supplemented with 100 ml mineral mix[7], 5 g KH_2PO_4, 150 g flowers of sulphur, 10 mg resazurin and 5 g Na_2S, *p*H 5.5 (H_2SO_4). It was gassed with a mixture of H_2/CO_2 (80:20 v/v, 6 l h^{-1}). Stirring was at 50 r.p.m. For protein determination, 850-ml portions were centrifuged (8,000 r.p.m., 30 min; WKF G 50 K centrifuge). The pellets were solubilized in 200 μl SDS (2% w/v) and boiled for 5 min. From this solution, protein was determined as described elsewhere[9]. For ATP determination, 1 ml of culture was centrifuged at 21,000 r.p.m. for 2 min in a J 21 Beckman centrifuge. The pellet was solubilized in 30 μl NRB solution (Lumac); 10 μl of this solution were mixed with 100 μl Lumit buffer (Lumac), 90 μl twice-distilled H_2O and finally with 100 μl enzyme (Lumit PM; Lumac). Then, the sample was immediately analysed with a scintillation counter (Biocounter M 2010; Lumac). As a reference, 1 ng ATP yielded 10,958 counts in the same conditions. For medium without inoculation, the background was only ~20 counts. The doubling time, determined by protein and ATP amplification, was 220 min. *b*, Growth in closed 25-ml serum tubes at various temperatures: (×) 85 °C; (○) 100 °C; (•) 105 °C. The tubes contained 10 ml culture medium as described above and were pressurized (2 bars) with H_2/CO_2 (80:20 v/v). The tubes were each inoculated with 0.5 ml of an overnight culture grown at the same temperature. Samples (1 ml) were withdrawn using a syringe and ATP determined as described above. The doubling times were 550, 220 and 110 min at 85 °C, 100 °C and 105 °C, respectively.

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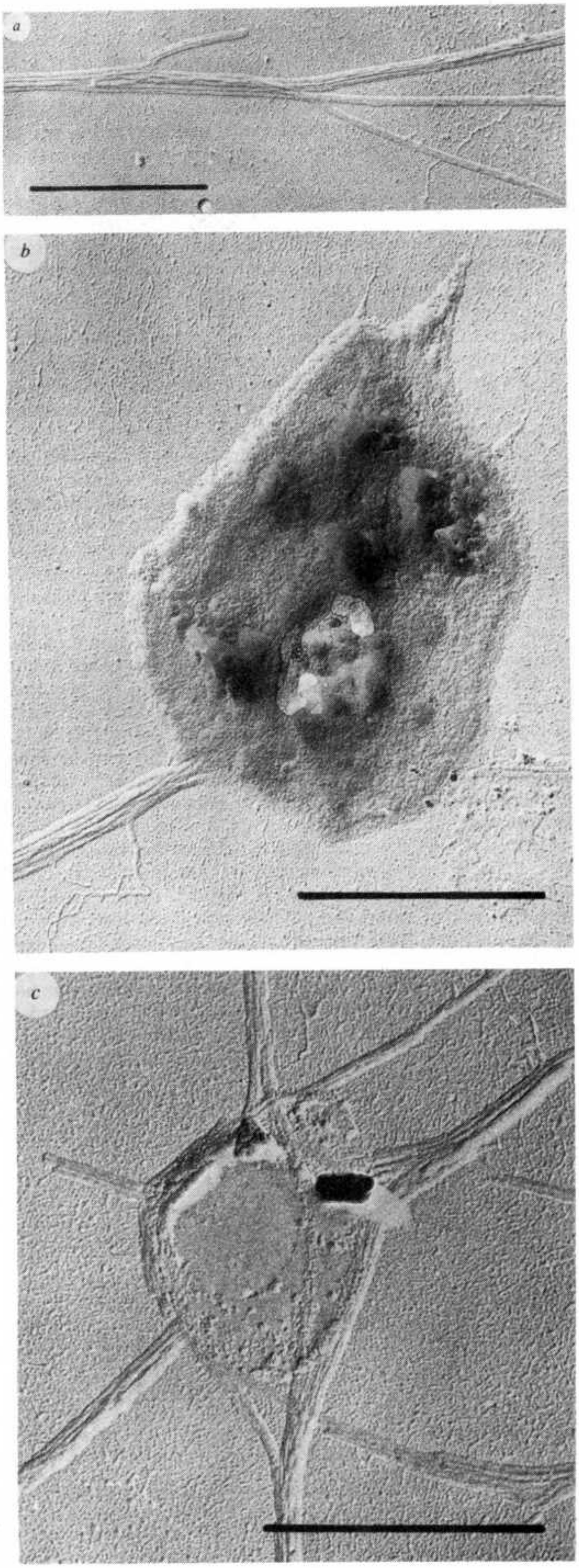

Fig. 2 Electron micrograph of isolate PL-19, platinum-shadowed. Scale bars, 1 µm. *a*, Bundle of hyphae; *b*, large disk-shaped cell situated laterally on a hypha; *c*, disk-shaped cell surrounded by hyphae.

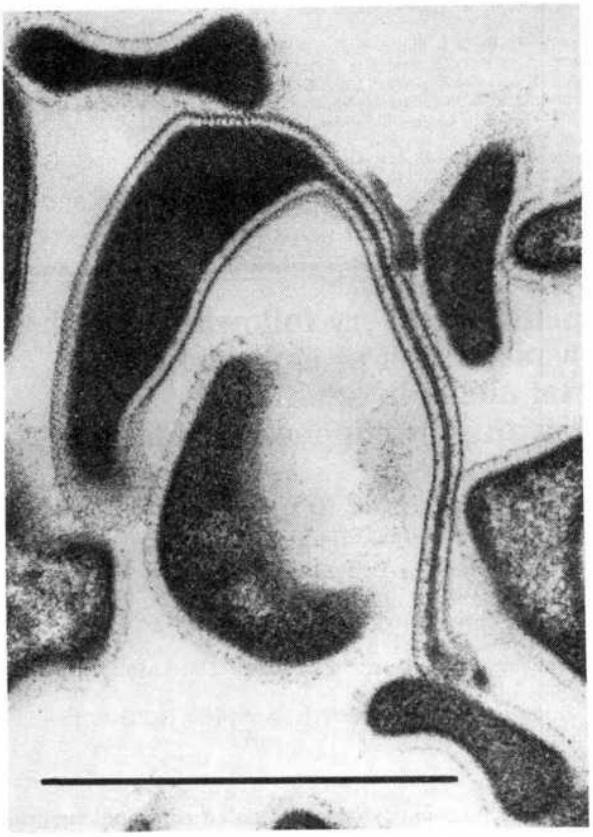

Fig. 3 Electron micrograph of thin sections of isolate PL-19. The sections were doubly contrasted with lead citrate and uranyl acetate. In the centre, a longitudinal section through a hypha attached to a disk-shaped cell is visible. Scale bar, 1 µm.

0.05–0.06 µm in diameter, thus they were not visible under the light microscope. The hyphae were often associated in bundles (Fig. 2*a*) or dispersed in loose networks covering areas of up to 10,000 μm^2. The disks were situated laterally (Fig. 2*b*) and terminally on the hyphae. Sometimes, disks appeared to be embraced by several hyphae (Fig. 2*c*). Thin sections revealed that both the disks and the hyphae were surrounded by a membrane and a protein envelope consisting of subunits ~30 nm in diameter (Fig. 3). The hyphae terminated within the disks without septa (Fig. 3); they formed only when the culture was grown without movement. Gentle stirring (200 r.p.m.) led to the formation of disks having no hyphae, and vigorous stirring (800 r.p.m.) led to rapid inactivation of the culture. Both the hyphae and the disks contained thin pili-like appendices (Fig. 2*a*,*b*) up to 5 µm long and 10 nm in diameter; occasionally these were branched, often cross-linking two individuals.

The taxonomic position of the novel organism described here is unclear. Preliminary studies of the membrane lipids (T. Langworthy, personal communication) and the existence of an ADP-ribosylable protein in the crude extract (F. Klink, personal communication) suggest that it may be a novel archaebacterium. As it is able to survive for long periods at low temperatures and due to its dependence on volcanic exhalations, this organism may be widely distributed in submarine volcanic areas.

After completion of this manuscript, Baross *et al.*[8] described the existence of bacterial communities in hot deep sea waters, which can grow at 100 °C in the laboratory and produce H_2, CH_4, CO and N_2O. The media used, however, contained no sulphur and neither H_2S formation nor growth at 105 °C was observed. Therefore, these organisms are very different from those reported here.

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Function recovery following neural transplantation of embryonic septal nuclei in adult rats with septohippocampal lesions

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The remarkable capacity of transplanted embryonic neurones to innervate the hippocampal formation of mature recipients has been well documented, with the pattern of innervation being shown to be anatomically specific and to resemble normal connectivity[1–3]. Although transplants are known to have functional consequences in other systems[4–9], information has yet to be obtained regarding the functional nature of embryonic septal transplants and the behavioural consequences of transplant innervation of the host hippocampal formation. We provide here evidence that a reinnervation of the hippocampal formation from cholinergic-rich septal transplants is functional in terms of the physiology of neural connectivity and that the newly formed connections can interact with an intrinsic afferent system, the perforant path. Moreover, we demonstrate that the reinnervation can aid in the partial recovery of the performance of a radial maze task that is thought to depend on the integrity of septohippocampal connections[10]. The behavioural performance of animals with transplants improved significantly compared with those without transplants when both groups were systemically injected with the acetylcholinesterase (AChE) inhibitor, physostigmine. These results suggest that neural transplants from embryonic tissue that reinnervate the hippocampal formation can form functional synaptic connections that can lead to the partial restoration of maze performance.

Each of three groups of female Sprague–Dawley rats (~180 g) received either bilateral lesions of the fimbria–fornix transecting all septohippocampal and commissural fibres followed by bilateral septal transplants (transplant group, $n = 8$), or similar bilateral lesions of the fimbria–fornix without transplants (lesion group, $n = 7$), or sham operations (control group, $n = 7$). The transplanted septal–diagonal band area was taken from 16–17-day-old embryos (crown–rump length 15–20 mm) of the same strain and implanted into the cavity created by the suction lesion of the fimbria–fornix. The cavity exposed a vascular bed overlying the anterior thalamus, upon which the embryonic tissue was placed as previously described[11]. To permit the transplant to reinnervate the hippocampal formation, the animals were allowed to survive for 7 months before behavioural testing.

The rats were tested with a raised eight-arm radial maze, as Olton *et al.*[10] have shown that the performance of this task is disrupted by damage to septohippocampal afferents. Rats were stabilized on a 23-h food deprivation schedule, which was maintained throughout behavioural testing. During each daily trial, each arm of the maze was baited and the rat was placed in the central arena, free to enter any arm in any order. Once the animal had entered an arm and consumed the pellet it received no food on subsequent visits to that arm. Optimal performance by the rat involved choosing only arms that had not previously been visited. The performance of each animal was determined by the number of correct arms (those arms that it had not previously visited during the trial) selected out of the first eight choices.

Analysis of maze performance during the first 30 days of testing revealed that the three groups differed significantly ($F = 4.93$ with 2, 19 d.f.). The control animals showed a gradual improvement over the first 15 days of testing and stabilized thereafter (linear trend over 30 days, $F = 12.64$ with 1, 54 d.f., $P < 0.001$). The performance of animals with bilateral lesions did not improve with repeated trials (analysis of linear trend not significant, $F = 0.02$ with 1, 54 d.f.). The animals with septal transplants, however, did improve over the 30 days of testing (linear trend $F = 4.26$ with 1, 63 d.f., $P < 0.05$), but their overall improvement was not as great as that of the control group and their overall performance did not differ significantly from the bilaterally lesioned animals.

We postulated that the lack of overall improvement in the transplant group when compared with the lesion group might be due to insufficient cholinergic innervation. To test this hypothesis, all animals were tested for an additional 8 days during which they were alternately injected (intraperitonally, i.p.) with 0.05 mg per kg of physostigmine, an acetylcholinesterase inhibitor, or with an equal volume of saline. Physostigmine has previously been shown to enhance discrimination performance[12,13].

Figure 1 shows the maze performance of the three groups under saline and physostigmine; the interaction between groups and drug was significant ($F = 4.13$ with 2, 18 d.f., $P < 0.05$). Both the control group and animals with transplants showed enhanced maze performance under the drug (planned orthogonal comparisons, $P < 0.005$ and $P < 0.001$, respectively) whereas the bilaterally lesioned group showed no improvement ($P > 0.25$). These results indicate that embryonic septal transplants can aid in the partial restoration of maze performance.

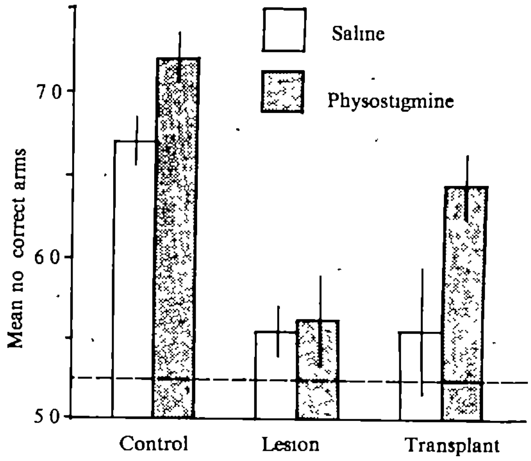

Fig. 1 Mean maze performance of rats with control sham operations ($n = 7$), fimbria–fornix lesions ($n = 7$) and septal transplants ($n = 8$) following injections of isotonic saline or 0.05 mg per kg physostigmine; vertical bars represent s.e.m. Both the control and transplant groups, but not the lesion group, showed significantly improved performance following injection of the cholinesterase inhibitor ($P < 0.05$, $P < 0.001$, and $P > 0.25$, respectively). The horizontal dashed line in each graph represents the random performance level.

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In particular, the enhanced maze performance with physostigmine administration in the transplant and control group, but not the lesion group, indicates that recovery is dependent on the re-formed cholinergic connections.

In addition to the extent of the cholinergic reinnervation, the complexity of the eight-arm maze task might affect the performance of the transplant and lesion groups. Roberts and Dale[14] have shown that rats tested with such a radial maze begin to adopt strategies when the memory requirements are quite demanding. Although animals of both groups tended to adopt strategies, the strategy generally adopted was to select alternate arms. This proved to be an unsuccessful strategy in that after the first four correct choices, rats would then enter arms that had been visited previously. Consequently, if an animal persisted in this strategy, it would err in its next four choices. Interestingly, animals in our control group did not have a tendency to adopt strategies, even successful strategies such as selecting adjacent arms as reported by Roberts and Dale[14]. When tested in maze tasks with less complexity, animals with transplants have been shown to perform significantly better than bilaterally lesioned animals without the aid of physostigmine[15].

On completion of the behavioural trials, five animals with septal transplants and one animal with fimbria–fornix lesion alone were used for electrophysiological analysis. The region of the dentate gyrus within the hippocampal formation was analysed because of the dense reinnervation in this area by the transplant as revealed by preliminary histological studies using AChE histochemistry. Furthermore, perforant path fibres from the entorhinal cortex also project to the granule cells of the dentate, and when electrically stimulated, evoke characteristic field potentials in the dendritic and somatic zones of the granule cell layer[16,17] (Fig. 2*a*, left panel).

The rats were anaesthetized with urethane (1.5 g per kg, i.p.) and placed in a stereotaxic apparatus where the transplant and surrounding neocortex were exposed and kept under saline. A field potential recording electrode was placed within the granule cell region of the dentate gyrus (anterior −3.5 mm, lateral 2.5 mm, vertical −2.7 to −3.7 mm with respect to bregma, incisor bar at −4.0 mm), and stimulating electrodes were placed on the transplant and in the angular bundle of the perforant path (anterior −8.1 mm, lateral −2.0 mm, vertical −2.0 to −3.0 mm). In all five animals studied with septal transplants, stimulation of the transplant resulted in positive-going monophasic field potential responses in the granule cell layer of the dentate (Fig. 2*a*, right panel, depth at 0 μm). The stimulation of the tissue surrounding the cavity in the lesioned animal without a transplant did not evoke field potential responses in the dentate.

Depth profile analysis of field potentials provided further evidence for the functional nature of the transplant innervation of the host. The depth profile (Fig. 2*a*) shows the field potentials evoked by alternating perforant path (left panel) and transplant stimulation (right panel) at successive depths during a single electrode penetration through the laminae of the dentate gyrus. The perforant path-evoked synaptic wave in the dentate exhibited a sharp polarity reversal as the electrode was moved from the dendritic zone down into the cell body layer. This characteristic reversal of the perforant path-evoked synaptic field potential in the dentate reflects the pattern of monosynaptic innervation from the perforant path which is confined to the mid and outer regions of the granule cell dendritic layer[18,19]. The transplant-evoked response during the same traverse continued to exhibit positive-going field potentials. Superimposed on these potentials were negative deflections which were observed when the recording electrode was situated in the dendritic region.

The innervation arising from the transplant was examined further to test its ability to interact with the fibre innervation arising from the perforant path. In this set of experiments, we set the stimulus intensity for the activation of perforant path fibres at a level that was insufficient to evoke the synchronous discharge of granule cells or population spike (Fig. 2*b*, trace 1). When the stimulation of the perforant path was preceded by the stimulation of the transplant, a prominent perforant path evoked population spike was observed (Fig. 2*b*, trace 2) which was maximal with inter-stimulus intervals in the range of 30–70 ms.

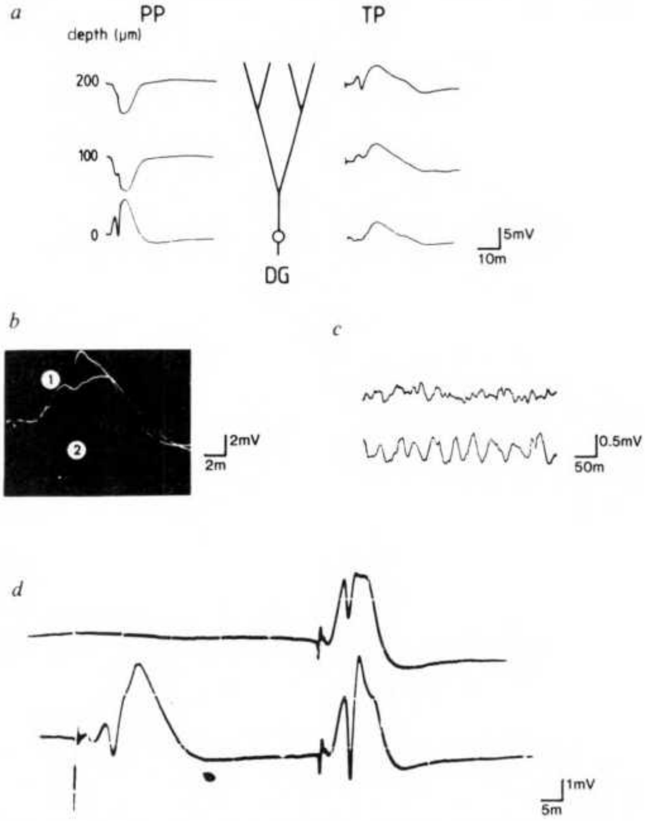

Fig. 2 Electrophysiology of dentate innervation. *a*, Vertical field potential profile of perforant path (PP) and transplant (TP) evoked responses through dentate laminae. Recording electrode positions relative to granule cell body layer (DG) are indicated. *b*, Subthreshold PP–dentate-evoked response (trace 1) and potentiated PP–dentate-evoked response (trace 2) potentiated by TP stimulation preceding PP stimulation by 50 ms. *c*, Monopolar recording of slow-wave activity (filters 0.1–100 Hz) from theta-generating zone of the dentate molecular layer 15 min after injection of physostigmine (0.05 mg per kg). Slow-wave activity in the theta-frequency range (6–10 Hz) is absent. An irregular pattern predominates (upper trace), interspersed with periods of regular sinusoidal activity at higher frequencies (15–20 Hz, lower trace). *d*, Field potentials from animal with intact septohippocampal connections. Trace 1: submaximal PP–dentate-evoked response. Trace 2: septo-dentate-evoked response (left) followed by potentiated PP–dentate-evoked response (right). All traces shown are positive up in polarity and calibrated individually.

Similar evoked responses and heterosynaptic interactions were observed in control animals with intact septohippocamporal connections. Stimulating electrodes were placed in the dorsal aspect of the medial septum and the angular bundle of the perforant path in rats anaesthetized with urethane (1.5 g per kg, i.p.). A recording electrode was placed in the granule cell layer of the dentate. Electrical stimulation of the perforant path alone evoked a population spike response as shown in trace 1 of Fig. 2*d*. When the stimulation of the perforant path was preceded by the stimulation of the dorsal aspect of the medial septum (50 ms interstimulus interval) the perforant path–dentate-evoked response was markedly potentiated (Fig. 2*d*, trace 2). The first response shown on trace 2 of Fig. 2 is the positive-going dentate field potential resulting from the stimulation of the medial septum. Superimposed on this is a negative deflection probably due to the synchronous activation of granule cells. The second response of trace 2 is the potentiated perforant path–dentate population spike. Septo-dentate-evoked responses have been demonstrated by other investigators[20], as well as heterosynaptic interactions within the hippocampal formation[21,22]. Note that in contrast to the presence of the negative-going septo-dentate spike as recorded in the granule cell layer of control animals, animals with septal transplants did not exhibit such spikes when the transplant was stimulated. The absence of the negative spike in these animals may be due to an insufficient convergence of fibres from the transplant required to synchronously activate the granule cells.

In the final set of electrophysiological experiments with the transplant group, we attempted to induce low-frequency (6–10 Hz) rhythmical electrical activity in the hippocampal formation (theta waves), which is thought to be paced by the medial septum[23,24]. Injections of physostigmine (0.05 mg per kg, i.p.) were capable of inducing rhythmical activity, but at frequencies higher than theta activity (Fig. 2*c*). The lack of theta activity may reflect an insufficient cholinergic reinnervation and/or the absence of other non-cholinergic afferents which were eliminated during the lesioning procedure. This, in turn, may account for the incomplete restoration of maze behaviour.

After the behavioural and electrophysiological studies animals were processed for AChE histochemistry. The animals with septal transplants, but not those with lesions alone, exhibited AChE-positive staining in the dorsal hippocampus with a laminar distribution typical of the cholinergic innervation in intact animals, as has been described in detail elsewhere[3].

Thus, the present study provides evidence that septal transplants are capable of forming physiologically viable connections with the hippocampal formation of the recipient. These connections, in turn, can enhance the efficacy of the perforant path input to the dentate gyrus. The cholinergic reinnervation, moreover, seems to account for the partial recovery of performance as examined with an eight-arm radial maze. The behavioural manifestations of the transplants, however, must also be viewed in the light of investigations demonstrating the ability of intracerebral transplants to become innervated by the host tissue[25–27]. Heterosynaptic interactions and interconnections indicate that the host and transplant are capable of integration in a physiological and anatomical sense. Such an integration provides a framework for the processing of neural information mediating the partial recovery of behavioural responses.

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Effector T lymphocyte line cells migrate to the thymus and persist there

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The thymus gland has been known for some time to be the central organ of differentiation of T lymphocytes[1,2]. Stem cells migrate into the thymus from the bone marrow, differentiate and, as competent T lymphocytes, disperse from the thymus to the periphery, where contact with specific antigen induces immune reactivity[2]. The traffic of T lymphocytes between the thymus and periphery has been thought to be unidirectional and, unless the cells are leukaemic[3] or the thymus gland has been irradiated[4], re-entry of peripheral T lymphocytes has not been detected[3]. We now report that cells from lines of functionally active T lymphocytes reactive to self or foreign antigens can migrate back into the normal thymus gland and persist there for relatively long periods in a quiescent state until activated by contact with antigen. Hence, in addition to being the seat of T lymphocyte differentiation, the thymus is open to two-way traffic with the periphery and may function as a repository of immunological memory.

The experiments described here were prompted by our investigation of the behaviour of cells of functional T lymphocyte lines. We have found that lines of rat T lymphocytes specific for the basic protein of myelin could cause experimental autoimmune encephalomyelitis (EAE) upon intravenous inoculation into naive recipient rats[5,6]. Moreover, cells from such lines, attenuated by treatment with mitomycin C or irradiation, could be used for vaccinating rats against the induction of active EAE by subsequent immunization of the rats to myelin basic protein[7,8]. To study the migrations of such cells, we labelled them with radioactive chromium-51, injected them into rats intravenously and measured the accumulation of radioactivity as an indication of the accumulation of the injected cells in various organs. We found that anti-myelin basic protein cells accumulated in the brain and spinal cord about one day before the onset of EAE (Fig. 1). Cells from lines reactive to other antigens, such as tuberculin (PPD), did not accumulate in the central nervous system. However, Fig. 1 shows that about 1% of injected anti-myelin basic protein or anti-PPD line cells accumulated in the thymus glands of recipient rats beginning 3 days after intravenous inoculation. Accumulation in the thymus was detectable only if the cells were intact and had been activated by incubation with specific antigen before inoculation. Non-activated cells did not enter the thymus, nor did activated and irradiated cells. It is noteworthy that nonactivated, or activated and irradiated cells also did not mediate EAE[7] or transfer delayed hypersensitivity (manuscript in preparation). Thus, migration to the thymus appeared to be correlated with the state of the T lymphocytes and their capacity to mediate specific immunological effects.

Experiments were then designed to answer three questions: (1) do the returned T lymphocytes persist in the thymus, (2) are they capable of responding to specific antigen and (3) do they include potential effector cells? We injected female anti-myelin basic protein or anti-PPD line cells into syngeneic male recipient rats, and two months later we assayed the proliferative responses of their thymus cells to myelin basic protein or PPD.

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The thymus glands used in the experiments were dissected free of the lymph nodes imbedded in their postero-lateral portions. The results are shown in Table 1. Control thymus cells from naive rats showed no response to myelin basic protein and only a weak response to PPD (SI = 2). The thymus cells of rats that had been inoculated intravenously with activated anti-myelin basic protein line cells and had recovered from EAE[8,11] responded weakly to myelin basic protein (SI = 3). The slight response to PPD of these thymus cells was about the same as that of naive control rats (SI = 2). Injection of anti-myelin basic protein cells did not produce a response to PPD. In contrast, thymus cells from rats that had been inoculated with activated anti-PPD cells showed a response to PPD (SI = 6) but not to myelin basic protein. Nonactivated anti-PPD cells produced no response.

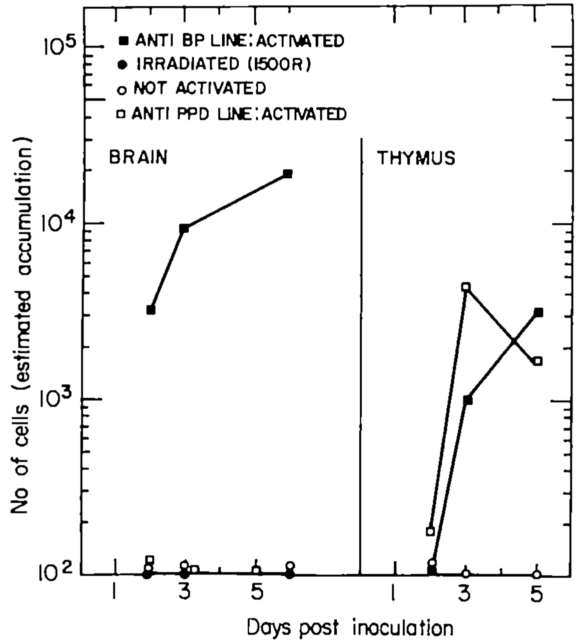

Fig. 1 Activated anti-myelin basic protein cells accumulate in the brain and thymus; activated anti-PPD line cells accumulate in the thymus. Lines of T lymphocytes specifically responsive to myelin basic protein or to PPD were raised from lymph node cells of 3-month old female Fischer rats that had been immunized with myelin basic protein-CFA[5,6]. The cells were maintained in medium containing T cell growth factor[9] and some of the cells were activated by incubation with their respective antigen, myelin basic protein or PPD in the presence of irradiated (1,500 rad) normal thymus accessory cells for 72 h (refs. 5, 6): some of these activated cells were irradiated with 1,500 rad. The line cells were labelled[10] by incubating 10^7 cells per ml Eagle's medium containing ^{51}Cr as sodium chromate (Amersham). The cells were washed by centrifugation in medium. Groups of 6 male Fischer rats were then inoculated into the tail vein with 3×10^6 labelled cells from lines of each type. On days 2, 3, 5 or 6, two rats of each group were killed and the accumulated line cells in the brains and thymuses were estimated by the c.p.m. emanating from each whole organ measured in a gamma counter. Differences between the organs of the two rats assayed at each time point were negligible. The number of cells was computed as the c.p.m. divided by the c.p.m. per cell in the inoculum. Similar results were obtained in four consecutive experiments.

To determine the immunospecificity of the cells responding to myelin basic protein and to determine their origin, they were grown in medium containing T-cell growth factor (IL-2)[9] with alternating periods of stimulation with myelin basic protein until they developed into a stable line of T lymphocytes[6]. Accessory cells added during stimulation with myelin basic protein were of male origin, as were the recipient rats from whom the thymuses were taken for culture. Thus, only the original line cells inoculated over 2 months earlier were of female origin.

Table 1 shows that cells recovered from anti-myelin basic protein lines responded strongly and specifically to the antigen *in vitro* (SI = 13), and mediated EAE upon intravenous inoculation into naive Fischer rats. Furthermore, analysis of the chromosomes of the recovered anti-myelin basic protein cells showed that they all had the female karyotype of the original line. Therefore, the antigen-specific cells detectable in the thymus 2 months after inoculation and spontaneous recovery from EAE were cells of the donor EAE effector line. Similar results were obtained in experiments using rats and line cells of the Lewis rat strain.

These findings indicate that competent, specifically sensitized T lymphocytes can re-enter the thymus and persist there. Thus, the thymus is connected to the periphery by a two-way flow of potential effector lymphocytes. The T lymphocytes that leave the thymus appear to be those induced to differentiate from precursors by contact with epithelial cells of the thymic stroma[13] or with macrophage-like cells[14]. This stage of differentiation is not dependent on clonal recognition of specific antigens but may involve the acquisition of the restriction of antigen recognition to association with particular products of the major histocompatibility complex[15,16]. In contrast, the T lymphocytes that return to the thymus from the periphery appear to be induced to do so in response to antigen. The cell lines used in these experiments were developed from cells of antigen-primed rats by their proliferative responses to specific antigens and by their ability to respond to T cell growth factor[5,6]. Furthermore, the cells did not return to the thymus and persist there unless

Table 1 Activated female anti-myelin basic protein T lymphocyte line cells persist in male thymus and can be recovered

Female line cells inoculated into male rats		Thymus cells		Recovered anti-myelin basic protein cells				
		Proliferation Δ c.p.m. (SI)		Proliferation Δ c.p.m. (SI)			Karyotype	
Specificity	Treatment	Myelin basic protein	PPD	Myelin basic protein	PPD	Incidence of EAE	Male	Female
None	None	95 (1)	921 (2)					
Anti-myelin basic protein	Activated	3,156 (3)	1,376 (2)	82,051 (13)	0 (1)	5/5	0/20	20/20
	Irradiated	0 (1)	880 (1)					
	Nonactivated	0 (1)	995 (2)					
Anti-PPD	Activated	0 (1)	4,984 (6)					
	Nonactivated	0 (1)	950 (2)					

Proliferative responses to myelin basic protein or to PPD of thymus cells from groups of five male 3-month old Fischer rats were measured[5,8]. Some of the groups had been inoculated i.v. 2 months earlier with 3×10^6 female anti-myelin basic protein or anti-PPD line cells[5,6]. Cells from the lines were either nonactivated, activated or activated and irradiated (see Fig. 1 legend). Rats that had been inoculated with anti-myelin basic protein line cells developed EAE 4 days later and recovered spontaneously[6,11]. An anti-myelin basic protein line was recovered from pooled thymuses free of lymphnodes of the male rats that had been inoculated with the female activated anti-myelin basic protein line cells[6]. The proliferative responses of these line cells to myelin basic protein and to PPD were tested[6], as was their ability to mediate EAE in naive recipient rats. The karyotype of the anti-myelin basic protein line cells was studied in 20 cells[12].

they had had contact with their specific antigen just before inoculation. Thus, the capacity of anti-myelin basic protein T lymphocytes to return to the thymus, to persist there and to mediate EAE appeared to be part of the programme of differentiation induced by the antigen. In their persistence and response to specific recall, these cells are a token of immunological memory.

We also have been able to recover EAE effector T lymphocytes from the thymuses of rats that had recovered from EAE induced by active immunization to BP in adjuvant[11]. Thus, the thymic immigration of effector T lymphocytes would seem to include cells generated endogenously as well as exogenous line cells. The anatomical site of residence in the thymus of the returned T lymphocytes is unknown, but it is probable that they comprise part of the pool of relatively mature longlived cells residing in the medulla[17].

The present observation raises a number of questions. Are all populations of sensitized lymphocytes represented in the thymus? Are some sensitized T lymphocytes specifically destined to return to the thymus because they bear particular receptors, or is the return a random process in which all sets of T lymphocytes participate equally? Does the persistence in the thymus of these returned cells have a role in immunological memory? Why and how are persistent autoimmune effector T lymphocytes kept quiescent? Is activation of such cells a factor in the progression or exacerbation of autoimmune diseases? Or, on the contrary, does the persistence of autoimmune cells help maintain self-tolerance by priming anti-idiotypic networks[18]?

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Human hybridomas secreting anti-islet autoantibodies

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Serum from patients with type I diabetes mellitus contains antibodies which react with all islet cells on sections of human pancreas[1], normal rat islet cell surface antigens[2], antigens expressed by islet cell tumours[3], β-cell-specific surface antigens[4] and A cells of sections of human pancreas[5]. Due in part to the low titre of anti-islet antibodies, the biochemistry of the relevant islet cell antigens is poorly understood[6]. Our laboratory has begun to develop and define a series of murine monoclonal anti-islet antibodies[7–9]. In addition, we have begun to explore the possibility of producing human hybridomas secreting monoclonal anti-islet antibodies by fusing lymphocytes from patients having type I diabetes mellitus with human myeloma cell lines. We describe here the isolation of human hybridomas that secrete human monoclonal anti-islet antibody. The availability of hybridoma clones secreting autoantibody should result in a better understanding of the mechanisms resulting in the expression of human autoantibodies.

After two unsuccessful fusions in which no anti-islet antibodies were detected, lymphocytes from 20 ml of blood from a patient with type I diabetes mellitus of 5 months' duration were fused using polyethylene glycol with the GM1500 6TG-2 myeloma-derived cell line (10^7 myeloma cells, $\sim 2\times 10^7$ lymphocytes)[10]. Using frozen sectioned pancreas and fluorescein-conjugated rabbit anti-IgG antibodies, the patient's serum reacted strongly with all islet cells in a typical anti-islet pattern, while with fluorescein-conjugated anti-IgM antibody it reacted selectively with peripheral islet cells, suggestive of an A-cell pattern[5]. After fusion, the resulting cells were distributed into 24 wells of a Linbro plate; colonies developed in 22 of the wells. Supernatants from these 22 hybrid cultures were tested for the presence of anti-islet antibodies reacting either with the cell surface of viable rat insulinoma cells or with islets of Bouin's fixed human pancreas. No supernatants contained an anti-cell-surface antibody.

Only one of the 22 hybrids, B6, produced an antibody which reacted with islets of Bouin's fixed pancreas. As shown in Fig. 1*b*, antibody B6, similarly to the anti-islet antibodies of patients with type I diabetes mellitus (Fig. 1*a*), reacts preferentially with islet cells of Bouin's fixed human type 0 pancreas. Preincubation of antibody B6 with insulin does not block its binding (Fig. 1*c*). Figure 1*d* is a fluorescein photomicrograph of a pancreas section incubated with antibody GM607, a negative control monoclonal IgM antibody (Fig. 2), as contrasted with the parental myeloma (IgG)[10]. We have subcloned the B6 hybrid cells by limiting dilution without feeder layers into microwells of 96-well Linbro plates. All six hybrid clones produced and secreted IgM ($\sim 0.4\ \mu g\ ml^{-1}$ of antibodies in spent culture supernatant) that reacted with islet cells in the same way as the parental B6 hybrid (Fig. 3). As all hybrid clones derived from B6 expressed anti-islet IgM, it is likely that hybrid B6 was itself a clone. The fact that all B6 clones were producing anti-islet IgM to the same extent (Fig. 3) and that B6 hybrids and its clones have maintained the production of the same amounts of anti-islet IgM after approximately 1 yr in tissue culture strongly suggest that such human–human hybridomas are quite stable. To

Table 1 HLA-antigen assignments

	A	B	C
GM 1500 myeloma	A2, Aw19 (?33)	B27, B18	Bw6
Patient	A2	B27, Bw16 (w39)	Bw4, Bw6
B6 cell line clone 10–13	A2, Aw19 (?33)	B27, B18, Bw16 (w39)	Bw4, Bw6

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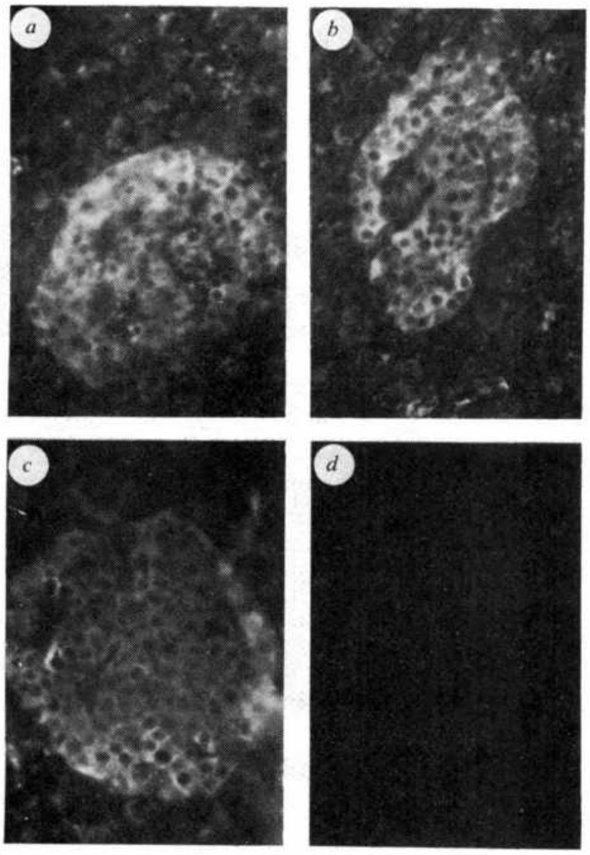

Fig. 1 Indirect immunofluorescence with Bouin's fixed human pancreas. Lymphocytes from 20 ml of heparinized blood from a child with type I diabetes were fused with the GM1500 6TG-2 human myeloma cell line using polyethylene glycol by methods previously described[10]. Supernatants from cultures were screened for antibodies reacting with the cell surface of a cloned rat insulinoma cell line using a ^{51}Cr-release assay[3], an ^{125}I-protein A radioassay[13] and fluorescein-coupled anti-antibodies in an indirect immunofluorescence assay. In addition, supernatants were incubated with 4-μm thick sections of Bouin's fixed human pancreas (type 0), and anti-islet antibodies were detected using fluorescein-coupled anti-human antibody (Miles). Supernatants from the GM607 (IgM) and the GM1500 (IgG) human myelomas were used as negative controls. Culture B6, which produces an anti-islet antibody, has been cloned by limiting dilution, and has stably produced antibody in culture for >1 yr. Antibody B6 is produced by cells grown in RPMI medium supplemented with 10% newborn calf serum, hypoxanthine (10^{-4} M) and thymidine (1.6×10^{-5} M). Antibody is collected from culture supernatants by adding an equal volume of saturated $(NH_4)_2SO_4$. The precipitate is resuspended in 1/20th its original volume of Dulbecco's phosphate-buffered saline (PBS) and then dialysed against PBS. Antibody B6 is then affinity-purified using rabbit anti-human antibody coupled to Sepharose. The antibody is eluted from this affinity column in 1-ml fractions with a glycine-HCl buffer (*p*H 2.28, 0.05 M glycine HCl, 0.15 M NaCl) neutralized with 20 μl of 1 M Tris and dialysed against PBS. *a*, Serum from a patient with type I diabetes; *b*, antibody B6; *c*, antibody B6 after incubation with insulin; *d*, control negative antibody GM607. ×188.

confirm that the cells producing antibody B6 are hybrid cells, the cell line B6 and the B6-derived positive clone 10-13, the patient's lymphocytes and the GM1500 parental cell line were HLA-typed by Dr Frances Ward (Duke University). As shown in Table 1, B6 and clone 10-13 cells contain the HLA antigens of both the myeloma-derived parent and the patient.

Anti-islet antibodies reacting with sectioned human pancreas are traditionally assayed using frozen sections of human pancreas[1] and only recently have Bouin's fixed sections been used to detect anti-islet antibodies[11]. We therefore studied the reaction of antibody B6 with frozen sections of human pancreas, both with and without acetone fixation (Fig. 4). Antibody B6 on frozen sections reacted only with cells that also stained with antibody to glucagon (A cells), whereas with Bouin's fixed sections, all islet cells reacted. Similar to results using anti-insulin antibody, double immunofluorescence with anti-somatostatin and anti-human pancreatic polypeptide antibodies showed no overlap of the cells reacting with antibody B6 (not shown).

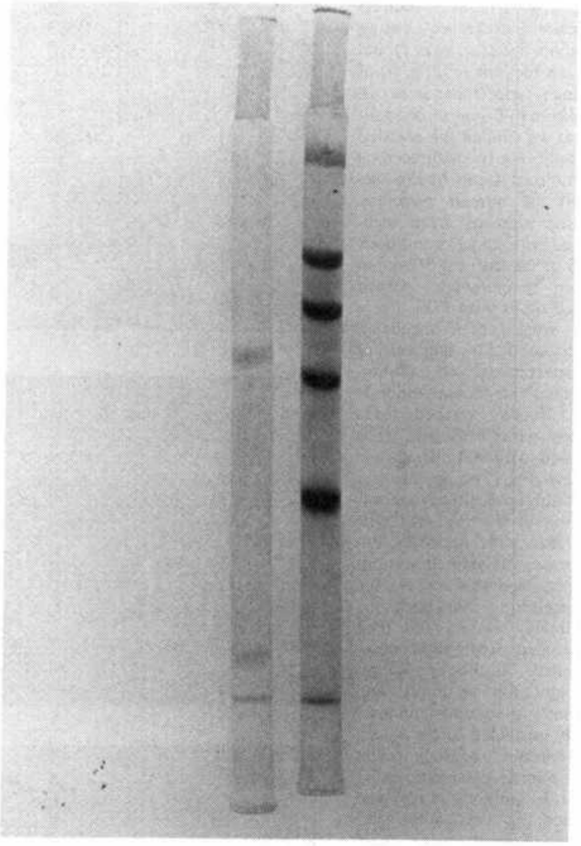

Fig. 2 10% Polyacrylamide gel run in reducing conditions with 10% SDS and stained with Coomassie blue (R250). Left gel, affinity-purified antibody B6; right gel, molecular weight standards (200,000; 116,250; 92,500; 66,200; 45,000). Heavy chain of antibody B6 has the molecular weight of an IgM heavy chain.

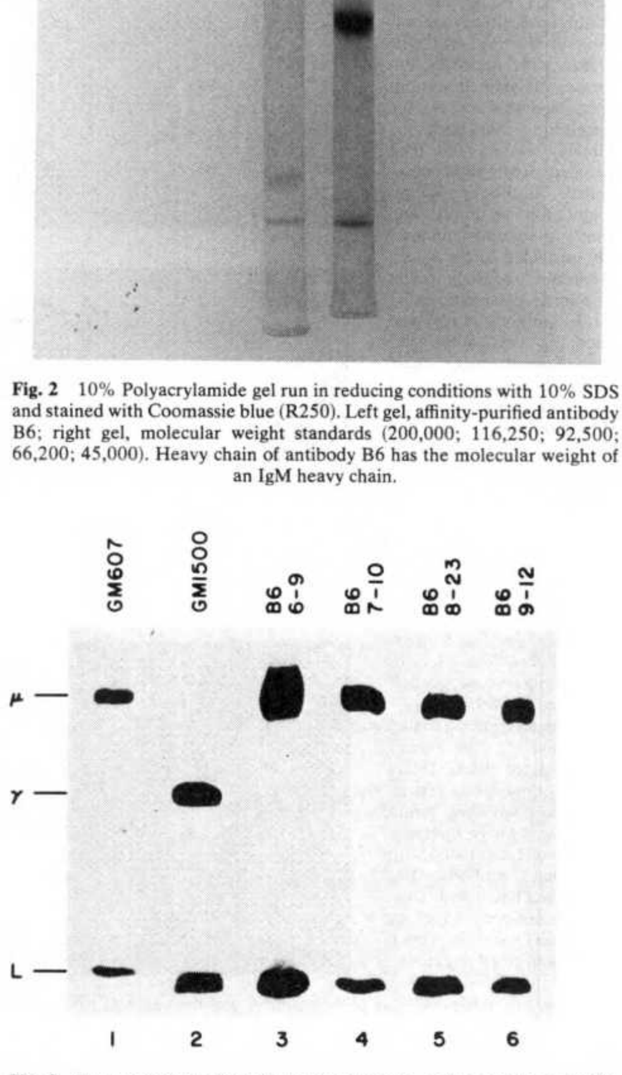

Fig. 3 Immunoprecipitation of immunoglobulin chains produced by B6 clones. The immunoprecipitation of the human immunoglobulin chains was carried out as described previously by using anti-human immunoglobulin rabbit antisera[10]. The human chains were separated by SDS-polyacrylamide gel electrophoresis[10]. Lane 1, human immunoglobulin chains secreted by GM607 human lymphoblastoid cells[10]; lane 2, human immunoglobulin chains secreted by the GM1500 human myeloma-derived parent; lanes 3–6, human immunoglobulin chains secreted by four clones of the B6 hybrid. Identical results were obtained with two additional clones and the parental B6 hybrid.

The technology for the production of murine and rat monoclonal antibodies is well developed, and such techniques have

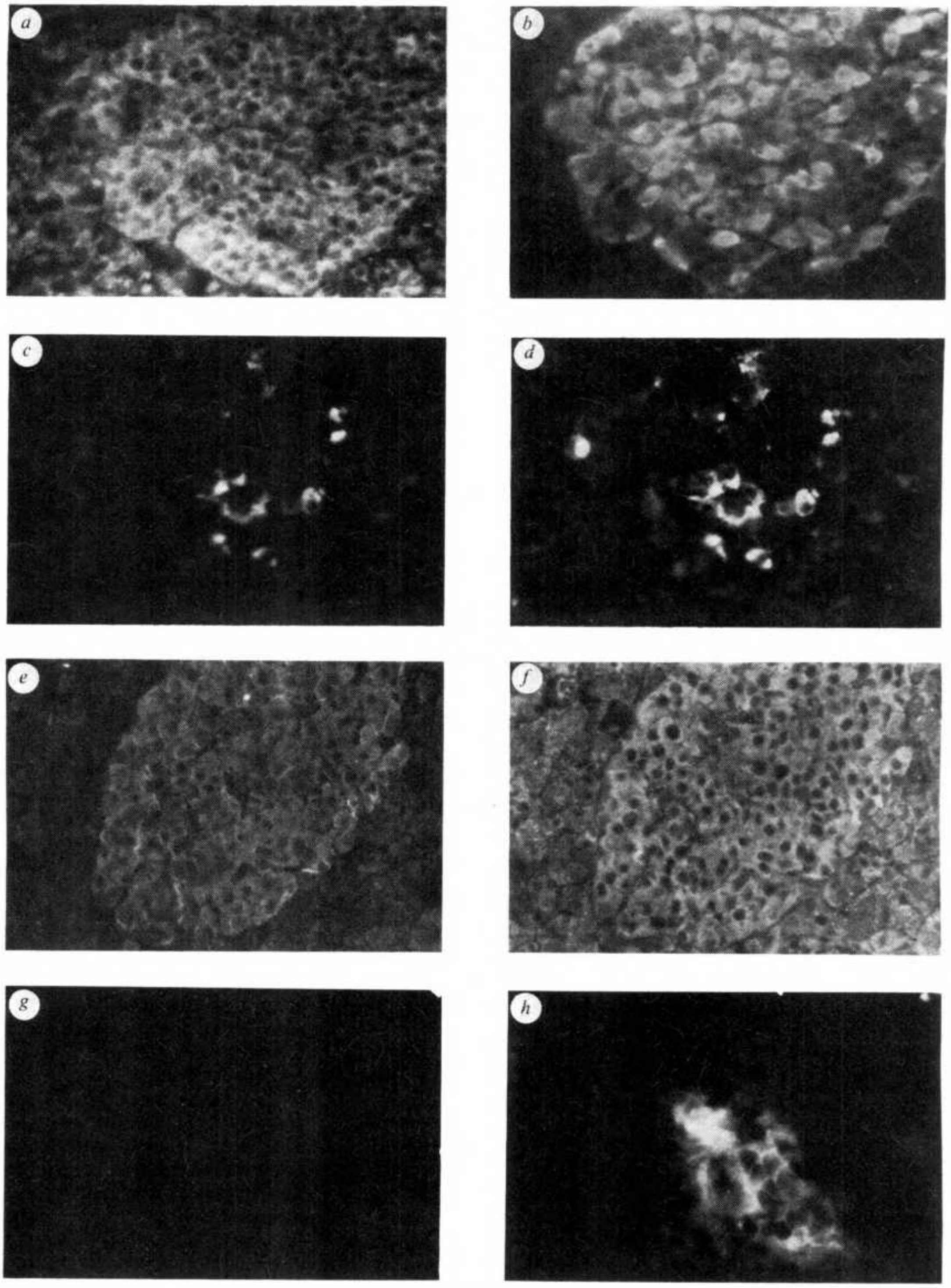

Fig. 4 Double indirect immunofluorescence using Bouin's fixed (*a*, *b*, *e*, *f*) and frozen sections (*c*, *d*, *g*, *h*) of human type 0 pancreas. In addition to Bouin's fixed pancreas we studied the reaction of antibody B6 with acetone or unfixed 4-μm frozen sections of human pancreas. Tissue sections were incubated with 25 μl of antibody (B6 or control) for 30 min at room temperature, washed three times with PBS (5 min per wash), then incubated with a 1:50 dilution of fluorescein-coupled affinity-purified anti-human antibody for 30 min, washed three times with PBS and then viewed with a Leitz diavert microscope equipped for epifluorescence. For determination of the islet cell type reacting with antibody B6, fluorescently stained sections were incubated with the appropriate anti-hormonal antibody, washed and then incubated with rhodamine-coupled antibody. Rabbit antiglucagon antibody and rabbit anti-somatostatin antibody (provided by M. Appel, Worchester, Mass.), rabbit anti-human pancreatic polypeptide antibody (Lilly) and guinea pig anti-insulin antibody (Cappel) were used. After a 30-min incubation at room temperature with the anti-hormonal antibody diluted 1:50 in PBS, 1% albumin, the sections were washed with PBS and incubated for an additional 30 min with a 1:50 dilution of appropriate anti-rabbit or anti-guinea pig rhodamine-labelled antisera. By sequentially viewing the section with the 'rhodamine' and 'fluorescein' fluorescent filters of our Leitz microscope, the cell type reacting with antibody B6 was determined. Human monoclonal antibodies [GM607 (IgM) and GM1500 (IgG)], normal rabbit serum, or normal guinea pig serum served as negative controls. Sections were incubated with monoclonal antibody B6, washed, incubated with antiglucagon antibody (*a*–*d*) or anti-insulin antibody (*e*–*h*), washed and then incubated with rhodamine-coupled anti-rabbit (*a*–*d*) or anti-guinea pig (*e*–*h*) antibody and then viewed with a Leitz microscope equipped for epifluorescence with either the fluorescein (*a*, *c*, *e*, *g* to detect B6 binding) or rhodamine filter (*b*, *d*, *f*, *h* to detect hormonal content). ×143.

been widely applied[12]. The production of human monoclonal antibodies by human–human 'hybridomas' has been more difficult. Ours is the first report of a human hybridoma producing a monoclonal autoantibody reacting with islet cells.

The pattern of reaction of antibody B6 with Bouin's fixed pancreas and frozen sections of pancreas is dramatically different. Bouin's fixation involves incubation of the pancreas with picric acid whereas frozen sections are simply air-dried or dipped in acetone before incubation with antibody B6. The qualitative difference in reaction pattern indicates that the method of pancreatic fixation can dramatically alter the interpretation of binding of even a single antibody. Such differences in reactivity indicate that the two techniques are not equivalent and may contribute to differences in clinical assays using the two methods.

Drs Bottazo and co-workers have described IgM antibodies in patients with type I diabetes and other individuals which bind to A cells on frozen sections[5]. Antibody B6 is an IgM monoclonal antibody which, on frozen sections, binds to A cells. As patients with type I diabetes express a series of anti-islet antibodies, the production of monoclonal antibodies with these specificities should now be feasible using the techniques which resulted in the generation of hybridoma B6. The ability to produce human monoclonal autoantibodies by simply fusing circulating lymphocytes provides a powerful tool for studying the autoimmune phenomenon of type I diabetes mellitus.

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A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor; partial characterization of the receptor

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T-cell growth factor (TCGF or interleukin-2) is an inducible glycoprotein hormone of molecular weight 15,000 (ref. 1) synthesized and secreted by T lymphocytes following activation with antigen or mitogen[2,3]. TCGF is required for proliferation and expansion of T cells following antigen encounter[4,5] and to maintain them in long-term culture *in vitro*[6–9]. Full expression of the human immune response requires both the induction of TCGF synthesis and the formation of specific TCGF membrane receptors[10,11]. Monoclonal antibodies binding TCGF have been prepared[12,13]. In contrast, antibodies specific for the TCGF membrane receptor have not been identified, nor has the receptor been characterized. We have prepared a monoclonal antibody, termed anti-Tac[14,15], which appears to bind to the human membrane receptor for TCGF. In support of this, we now demonstrate that anti-Tac suppresses TCGF induced proliferation of T cells and blocks binding of radiolabelled TCGF to cells from a cloned human continuous T-cell line. Also we have partially purified and characterized the putative TCGF receptor. This receptor is a glycoprotein with a molecular weight (M_r) of 47,000–53,000.

Monoclonal anti-Tac was used in both ascites and Protein A–Sepharose purified[16] forms. Two human continuous T-cell (CTC) lines, CTC-2 and HUT–102B2, were used in these studies. CTC-2 is TCGF dependent and derived from the peripheral blood of a patient with a nonleukaemic cutaneous T-cell lymphoma. CTC-2 is a subclone of CTC-1 and has been in culture for more than 2 yrs. HUT–102B2 is a cloned CTC line established from a lymph node of the same patient[17]. In contrast to CTC-2, HUT–102B2 constitutively produces sufficient endogenous TCGF so that exogenous TCGF is not required. Cells from this clone express membrane TCGF receptors[18]. As recently reported by Poiesz and co-workers[19], HUT–102B2 cells contain and shed type C retrovirus. Cells from both CTC lines formed rosettes with sheep erythrocytes, reacted with OKT3 monoclonal antibody (a pan-T-cell-specific reagent)[20], and did not exhibit detectable surface immunoglobulin or Epstein–Barr nuclear antigen.

We initially looked at the effect of anti-Tac on TCGF-induced proliferation of CTC-2 cells. As shown in Fig. 1*a*, anti-Tac ascites markedly inhibited TCGF-induced ^{3}H-thymidine incorporation at final dilutions of 10^{-2}–10^{-4}, whereas equivalent concentrations of control ascites had no significant effect. Purified anti–Tac antibody also inhibited proliferation of CTC-2 cells, but purified monoclonal anti-Ia, though binding to these cells, had no effect (Fig. 1*b*). In agreement with these data, G. Bonnard and co-workers have independently demonstrated anti-Tac inhibition of TCGF induced proliferation of other human CTC lines (personal communication). In contrast, anti-Tac did not inhibit the growth of TCGF independent T-cell lines (CEM, HUT–78) or Epstein–Barr virus-transformed B-cell lines including a B-cell line derived from the same patient as the CTC lines.

All these experiments were performed in the absence of complement and anti-Tac did not produce cell death measured by short-term (4 h) ^{51}Cr -release assays or long-term (24–48 h) supravital dye exclusion[21] studies. In addition, the inhibitory effect of anti-Tac on proliferation of CTC-2 cells was reversible. CTC-2 cells precultured for 18 h with inhibitory concentrations of anti-Tac (10^{-3} dilution of ascites) incorporated thymidine in amounts equivalent to untreated cells following removal of cell-associated antibody by thorough washing and stimulation with TCGF.

We next investigated whether anti-Tac inhibited TCGF-induced proliferation by blocking the interaction of TCGF with its membrane receptor. ^{3}H-TCGF was prepared by phytohaemagglutinin induction of TCGF synthesis in a cloned human T leukaemia cell line (JURKAT)[22] in the presence of ^{3}H-lysine and ^{3}H-leucine. Radiolabelled TCGF produced in the absence of serum was purified by ultrafiltration and molecular sieve chromatography as previously described[10], and produced a single spot when evaluated by two-dimensional gel electrophoresis. As shown in Fig. 2, preincubation of HUT–102B2 cells with anti-Tac at concentrations greater than 3 μg ml^{-1} produced nearly complete blockade of purified ^{3}H-TCGF binding in experiments performed at 37 °C. In contrast, while binding to these cells, similar amounts of monoclonal anti-Ia did not alter ^{3}H-TCGF binding. The dose–response relationship

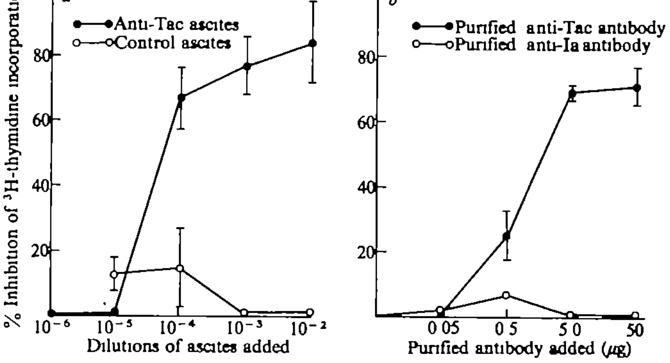

Fig. 1 Anti-Tac blocks TCGF induced proliferation of T cells from a human CTC line. CTC-2 cells were washed four times in a balanced salt solution and suspended at 0.5×10^6 cells per ml in RPMI 1640–10% heat-inactivated fetal calf serum. Aliquots (0.2 ml) of this cell suspension were distributed into wells of flat-bottom 96 well microtitre plates and preincubated with varying concentrations of antibodies for 30 min at room temperature. *a*, Data obtained with dilutions of heat-inactivated anti-Tac and control ascites (SPQC 11, from Dr M. Potter); *b*, data obtained with Protein A-Sepharose-purified anti-Tac and DEAE-purified monoclonal anti-Ia antibodies. Crude TCGF was subsequently added at a final concentration of 10% v/v. Triplicate cultures were incubated for 48 h at 37 °C in a humidified atmosphere containing 5% CO_2. Four hours before the end of culture, 1.0 μCi of ^{3}H-methyl-thymidine was added to each well followed by transfer of cells onto fibreglass filters with a multiple channel automated cell collector. Cell-associated radioactivity was measured by liquid scintillation. Data are expressed as % inhibition of the response obtained in cultures receiving TCGF alone (28,288±784 c.p.m.).

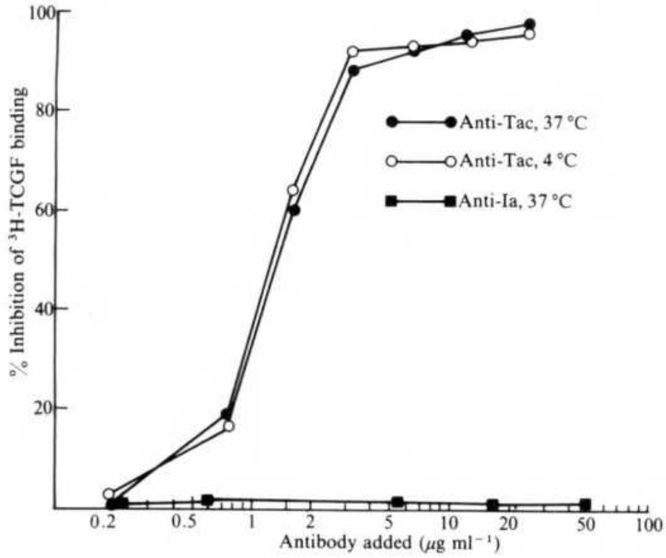

Fig. 2 Anti-Tac blocks the binding of ^{3}H-TCGF to HUT–102B2 cells. HUT–102B2 cells were washed three times in RPMI 1640 and resuspended at 1.1×10^7 cells per ml in RPMI 1640 containing 25 mM HEPES and 2 mg per ml bovine serum albumin. Cells and varying amounts of purified anti-Tac or anti-Ia antibodies were incubated together for 90 min at 37 or 4 °C in a rotating water bath. Following this preincubation, 50 pmol of purified ^{3}H-Leu, ^{3}H-Lys TCGF were added and the reaction was continued for an additional 20 min at 37 °C or 60 min at 4 °C. Cells were then pelleted, resuspended in 100 μl of RPMI 1640, and centrifuged (9,000*g* for 90 s) through cushions of 84% silicone and 16% paraffin oil to remove unbound ^{3}H-TCGF. Microfuge tube tips were excised, placed in liquid scintillation vials, and the cells solubilized by addition of 100 μl 1% SDS and 3 ml of liquid scintillation mixture followed by measurement of cell-associated radioactivity. Each experiment was performed in duplicate. Data shown are from two different experiments. A third experiment performed at 37 °C produced similar results.

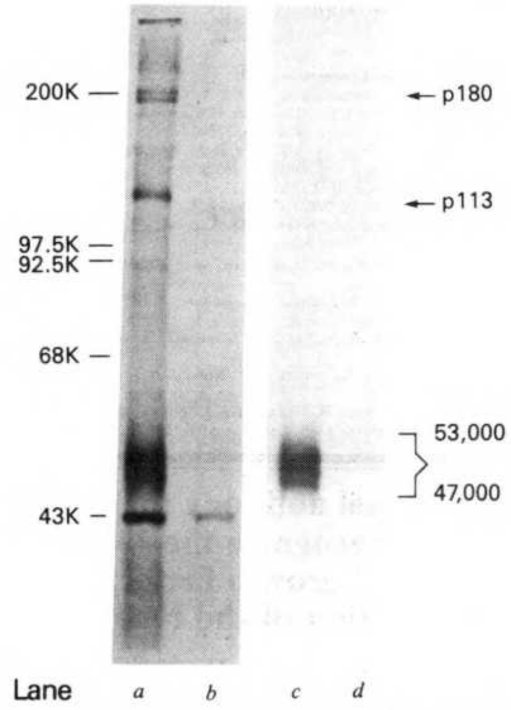

Fig. 3 Purification of the anti-Tac membrane receptor. Five million HUT–102B2 cells were washed twice in a balanced salt solution and suspended in 5 ml of methionine-free Selectamine medium (Gibco) containing 5% dialysed fetal calf serum. 0.3 mCi of NEN translation grade ^{35}S-methionine (>800 Ci mmol^{-1}) was added and the cells were cultured overnight at 37 °C. Cells were washed once and extracted in 0.14 M NaCl, 10 mM Tris *p*H 7.5, 1% Triton X-100, and 100 μg ml^{-1} phenylmethylsulphonyl fluoride (PMSF) for 30 min at 4 °C. The supernatant of a 60-min spin at maximum speed was then immunoprecipitated as follows. The supernatant was cleared once with 5 μl of control ascites (NS1, a non-secreting ascites provided by Dr Jay Berzofsky, NIH), 5 μl of RPC5 (a control IgG2a-k antibody, Litton Bionetics), and 50 μl of 10% w/v formaldehyde fixed Cowan I strain staphylococci and then once with 50 μl of the staphylococcal organisms alone. The supernatant was immunoprecipitated with 2–3 μg of protein A sepharose purified anti-Tac or UPC10 (a second control IgG2a-k antibody, Litton Bionetics) for 20 min at room temperature followed by a 20 min incubation with 20 μl of Cowan I strain staphylococci at room temperature. The immunoprecipitate was resuspended in a buffer containing 0.1% SDS, 0.5% NP40, 0.2% deoxycholate, 100 μg ml^{-1} PMSF and washed twice through the same buffer containing 1M sucrose. Final pellets were boiled in 1% SDS in the presence or absence of 0.1M dithiothreitol and analysed on 7.5% discontinuous SDS-polyacrylamide gels. Gels were intensified and fluorographed for 24 to 48 h at −195 °C. Analysis under reducing conditions is shown. *a* and *b* show the electrophoretic patterns obtained following immunoprecipitation of ^{35}S-methionine incorporated cells with anti-Tac and UPC10, respectively. The bands at 200,000 (200K) and 43,000 (43K) co-migrate with myosin and actin, respectively. Migration of molecular weight standards is shown on the left. The locations of p180, p113, and p50 are indicated on the right. *c* and *d* represent electrophoretic patterns obtained after immunoprecipitating surface-labelled cells with anti-Tac and UPC10, respectively. Approximately 5–10×10^6 cells were washed and suspended in 100 μl of a balanced salt solution and 1 mCi carrier-free ^{125}I was added. 4 μl lactoperoxidase solution (1U per 10 μl) and 7.5 μl H_2O_2 solution (0.03%) were then added and cells incubated for 4 min at room temperature. An additional 2 μl of lactoperoxidase solution and 7.5 μl H_2O_2 were added and cells incubated for an additional 4 min. Cells were then washed in a balanced salt solution and immunoprecipitated as described above.

for anti-Tac inhibition of TCGF-induced CTC proliferation and blockade of TCGF binding were similar in several experiments. As shown in Fig. 2, anti-Tac also inhibited ^{3}H-TCGF binding at 4 °C. These data argue that anti-Tac inhibition of TCGF binding is not simply secondary to receptor capping or co-capping with subsequent receptor endocytosis or shedding, because these rearrangements are largely inhibited at 4 °C (ref. 23).

Studies of ^{125}I-anti-Tac binding to HUT–102B2 cells at 20 °C revealed half saturation of available receptor sites within 3.75 min of anti-Tac addition. Next, ^{125}I-anti-Tac was incubated with HUT–102B2 cells at 4 °C in the presence of 0.1% sodium azide to block cap formation and energy-dependent endocytosis. After 1 h, steady-state binding was achieved. Excess unlabelled antibody was then added and reversible binding was demonstrated as 90% of the bound radiolabelled anti-Tac molecules were shown to dissociate. Glutaraldehyde fixed HUT–102B2 cells bound ^{125}I-anti-Tac in amounts slightly less than non-fixed cells. Preliminary Scatchard analysis[24] of anti-Tac binding with HUT–102B2 cells indicates ~100,000 high-affinity receptors per cell. This receptor number is 7- to 8-fold higher than the number of TCGF receptors reported by Robb *et al.*[10]. This difference may in part be accounted for by different experimental binding conditions and slightly different cell lines (our HUT–102B2 line is not 6-thioguanine resistant in contrast to that of Robb and co-workers). Anti-Tac had a K_d of $\sim 10^{-9}$ mol l^{-1} suggesting a much lower affinity than that reported for TCGF ($K_d \sim 10^{-12}$ mol l^{-1})[10]. Thus, the preincubation with anti-Tac before addition of ^{3}H-TCGF may have been critical to our ability to perform the successsful competitive binding experiment (Fig. 2).

Finally, we have partially purified and characterized the membrane receptor recognized by anti-Tac. SDS-polyacrylamide gel electrophoretic analysis of this receptor from HUT–102B2 cells is shown in Fig. 3. In both reducing (shown) and nonreducing (not shown) conditions, two major proteins with molecular weights of approximately 113,000 and 47,000–53,000 were precipitated by anti-Tac antibodies from cells internally labelled with ^{35}S-methionine (*a*). A third minor band with an M_r of approximately 180,000 was also identified. For convenience, we will denote these bands p113, p50, and p180, respectively. None of these bands was identified in immunoprecipitations using control UPC10 IgG2a-k antibodies (*b*). Further, similar bands have been found in immunoprecipitations performed on phytohaemagglutinin stimulated peripheral blood lymphocytes from a normal individual (data not shown), thereby providing evidence that none are related solely to viral products. To determine whether any of these proteins are located on the outer cell membrane, similar anti-Tac and UPC10 immunoprecipitations were performed using cells that were surface iodinated with the lactoperoxidase method (*c* and *d*). Only p50 was immunoprecipitated with anti-Tac. We therefore hypothesize that this protein represents the actual binding site for anti-Tac. This protein can be identified in immunoprecipitations from cells incorporated with ^{3}H-D-glucosamine (Fig. 4), thus demonstrating that it is a glycoprotein, as is usually the case for membrane receptors. The roles of p113 and p180 are unknown. One or both may be part of a theoretical receptor complex, and therefore coimmunoprecipitated because of strong hydrophobic interactions with p50. It is also possible that neither has any functional relation to the receptor identified by anti-Tac, but rather that they contain antigenic determinants that result in their recognition by anti-Tac.

In summary, we have prepared a monoclonal antibody which blocks the binding of purified ^{3}H-TCGF to human CTC cells and inhibits TCGF induced proliferation of CTC cells, and which we hypothesize binds to the TCGF receptor. The receptor recognized by the antibody is a glycoprotein of molecular weight ~47,000–53,000. In recent studies with human peripheral blood T cells, we have also found that anti-Tac inhibits (>80%) soluble or alloantigen induced T-cell proliferation as well as the generation of cytotoxic T cells in allogeneic cell cocultures. Further, anti-Tac suppresses immunoglobulin production in cultures of peripheral blood lymphocytes stimulated with pokeweed mitogen, a T-cell dependent polyclonal activator. Thus, anti-Tac may permit not only further characterization of the human TCGF receptor but in addition may allow analysis of the site(s) and mechanism of TCGF action in both the cellular and humoral phases of the human immune response.

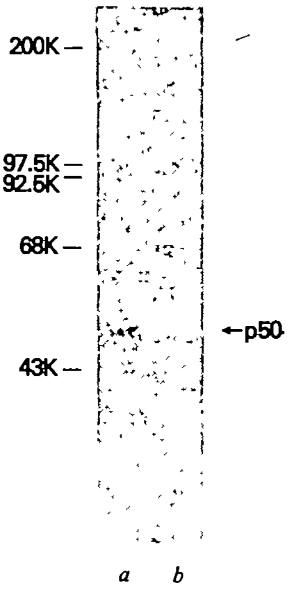

Fig. 4 The anti-Tac membrane receptor is a glycoprotein. 5×10^6 HUT-102B2 cells were washed once in a balanced salt solution and resuspended in RPMI media containing 5% fetal calf serum. Two hundred microcuries of D-[1,6-^{3}H(*N*)]-glucosamine HCl (NEN, 32.5 Ci mmol^{-1}) were then added and cells incubated for 4 h at 37 °C. Cells were washed once in balanced salt solution and then extracted with 0.14 M NaCl, 10 mM Tris-HCl *p*H 7.5, 1% Triton X-100, and 100 μg ml^{-1} PMSF for 30 min on ice. The extracts were then immunoprecipitated with anti-Tac (*a*) or UPC10 (lane *b*) as described in Fig. 3 legend, and then electrophoresed on 7.5% SDS gels and autoradiographed. Molecular weight markers are indicated on the left.

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Luteinizing hormone release from dissociated pituitary cells by dimerization of occupied LHRH receptors

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Studies of luteinizing hormone-releasing hormone (LHRH) have revealed several structural requirements for its biological activity[1]. Shortening of the decapeptide leads to loss of activity; positions 1, 6 and 10 are regarded as important in that they have a high affinity for the hormone receptor, and positions 2 and 3 (His-Trp) are thought to comprise the active centre of the molecule (replacement of His or Trp produces the most effective antagonists). Using a potent agonist analogue of the decapeptide, <Glu-His-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-NHEt, coupled to ferritin, we have demonstrated previously the binding, aggregation and internalization of bound hormone using dissociated pituitary cells[2]. Similar processes occur for other biologically active peptides, for example, insulin[3] and epidermal growth factor (EGF)[4]. Using a pure antagonist in the simpler LHRH system we now show that the N-terminal portion of the molecule is important for receptor aggregation and that dimerization of occupied LHRH receptors is a necessary early stage in the release of luteinizing hormone.

The octapeptide, Z-Gln-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NHEt, is an effective inhibitor of LHRH both *in vitro* and *in vivo*[5]. We prepared the 6-D-Lys analogue of this peptide to allow easy substitution at a central position in the molecule;

Table 1 Secretion of luteinizing hormone from porcine pituitary cells in culture

Peptide	Antiserum	LH released
LHRH (10^{-9} M)		185±24
LHRH (10^{-8} M)		300±34
Inhibitor (10^{-9} M)		29±5
Inhibitor (10^{-6} M)		49±5
LHRH (10^{-9} M) + inhibitor (10^{-6} M)		35±12
Inhibitor (10^{-8} M)	Rabbit anti-inhibitor (1:1,000 dilution)	215±36
Inhibitor (10^{-6} M)	Rabbit anti-inhibitor (1:1,000 dilution)	112±16
Inhibitor (10^{-8} M)	Rabbit anti-D-Lys agonist (1:1,000 dilution)	29±8
LHRH (10^{-8} M)	Rabbit anti-inhibitor (1:1,000 dilution)	361±51

Cells were incubated at 37 °C for 24 h after dissociation at 4×10^6 cells per 2.5-cm diameter Petri dish; before each experimental incubation they were washed three times in serum-free medium. The cells were then incubated in 1 ml medium for 30 min with additions as shown. Values are ng $\mathrm{ml^{-1}}$ ± s.e.m. luteinizing hormone.

this in turn would allow the formation of active macromolecular conjugates with the agonist[2]. The peptide was prepared from the protected heptapeptide described previously[2] (H-Trp-Ser-Tyr($\mathrm{Bu^t}$)-D-Lys(BOC)-Leu-Arg-Pro-NHEt). This was coupled, in dimethylformamide, with the activated derivative Z-Gln-OCP, and the product purified by chromatography on Sephadex LH-20 in methanol. The side chain protecting groups were removed with trifluoroacetic acid/water (9:1) and the peptide was isolated by chromatography on Sephadex G-50 in acetic acid/water (1:4). Analysis of an acid hydrolysate showed good amino acid ratios and the peptide was revealed as a single spot by high voltage electrophoresis at *p*H 2.1, 6.5 and 8.9 using ninhydrin, Ehrlich's and Sakaguchi visualization reagents and by TLC on silica gel plates in *n*-butanol/acetic acid/water (4:1:5).

The pure peptide was then coupled through the side chain of the central Lys residue to bovine serum albumin (BSA) using 1-ethyl-3(3-dimethylaminopropyl)carbodiimide. Antisera to the peptide were raised in rabbits by injection of conjugate (1.0 mg) in Freund's complete adjuvant subcutaneously (s.c.), followed by two booster injections of conjugate in saline at intervals of 4 weeks. The antisera were able to bind iodine-labelled LHRH, indicating that there were features common to the hormone and antagonist.

Dissociated porcine pituitary cells were prepared as described previously[6] and luteinizing hormone (LH) release measured by radioimmunoassay for the various treatments (Table 1). Clearly, the 6-D-Lys analogue of the antagonist[5] retained similar activity *in vitro* as gonadotropin release by the native hormone was suppressed completely at concentrations 100 times that of the agonist. The analogue alone gave no gonadotropin release at concentrations up to 10^{-6} M, indicating that the occupied receptor complex lacked the structure required for a biological response. However, the presence of antibodies converted this antagonist complex into a functional agonist by aggregating occupied receptors as a first stage. Lower levels of gonadotropin were secreted at higher concentrations of peptide, presumably because of extensive binding of antibody by free peptide in solution. The specificity of the effect was demonstrated by the fact that the antiserum raised against the peptide having <Glu-His instead of Z-Gln was unable to evoke a response. Although this indicated that antibody binding occurred through the N-terminal region, the anti-inhibitor antiserum may have multiple specificity and could also contain different immunoglobulin isotypes. Thus, although aggregation is involved, its scale and specificity is unclear.

From the rabbit antiserum to the inhibitory peptide, we prepared pure IgG having specificity for the N-terminus of the peptide (for experimental details see Table 2). The purified specific IgG and Fab proteins were then used to study LH release from pituitary cells (Table 2).

For this particular cell preparation, LHRH at 10^{-7} M stimulated LH release 3–4-fold. The inhibitor alone was ineffective at 10^{-9} M but in conjunction with purified specific IgG at $\sim10^{-11}$ M, LH release occurred. That this required divalency was apparent from the inability of similar amounts of Fab fragment to evoke similar effects and by the ability of goat anti-rabbit anti-serum to induce the Fab–antagonist complex to stimulate LH release. The effect of all agents together (Table 2) indicated that LH release was only mediated via the LHRH receptor.

Previously, we have observed aggregation and internalization of bound LHRH[2] and it has been shown that receptor aggregation is essential for the action of some polypeptide hormones. Thus, antibodies to the insulin receptor produced insulin-like responses directly[7,8] and a degraded EGF analogue became

Table 2 Sequential experiments with porcine pituitary cells in culture showing LH release for each treatment period.

Control period	Period 1		Period 2	
25.0±2.4	LHRH (10^{-7} M)	94.4±4.7		
30.3±4.2	Inhibitor (10^{-9} M)	28.2±1.8		
30.5±1.8	Inhibitor (10^{-9} M) + 1.3 ng $\mathrm{ml^{-1}}$ IgG	78.4±4.3		
20.0±4.7	Inhibitor (10^{-9} M) + 0.13 ng $\mathrm{ml^{-1}}$ IgG	26.4±2.6		
30.5±3.73	Inhibitor (10^{-9} M) + 0.8 ng $\mathrm{ml^{-1}}$ Fab	37.6±8.3	Inhibitor (10^{-9} M) + 0.8 ng $\mathrm{ml^{-1}}$ Fab + GaRS (1:20)	150±20.3
23.6±2.8	Inhibitor (10^{-9} M) + 0.8 ng $\mathrm{ml^{-1}}$ Fab + LHRH (10^{-7} M)	120.5±6.2	Inhibitor (10^{-9} M) + 0.8 ng $\mathrm{ml^{-1}}$ Fab + LHRH (10^{-7} M) + GaRS (1:20)	126.4±16.2

Pituitary cells were prepared and plated out as described in Table 1. Between each 30-min incubation period, the cells were rinsed with serum-free medium for three 1-min periods. Values are ng $\mathrm{ml^{-1}}$ ± s.e.m. luteinizing hormone. The goat anti-rabbit serum (GaRS) had no significant effect on LH release when used alone at 1:20 dilution. The purified specific IgG and Fab proteins used in the study were prepared as follows.

Rabbit antiserum (1 ml) to the inhibitory peptide, diluted with phosphate-buffered saline (PBS), was recycled through an affinity column comprising 2 ml Sepharose coupled to 20 mg of the C-terminal peptide-sequence (Leu-Arg-Pro-NHEt) at 4 °C for 48 h. The non-retarded serum solution was recycled through a 5 ml column of Sepharose–Protein A (Pharmacia) for 24 h. The column was washed with 5 vol PBS, eluted with 5 ml 0.1 M citrate buffer, *p*H 3.5 and the eluate neutralized immediately with 0.5 ml M Tris-HCl buffer, *p*H 8.0. The lyophilized eluate was taken up into water and applied to a column of Ultrogel ACA 34 (60×0.9 cm) in 0.05 M ammonium bicarbonate. The fractions from the single peak in the precalibrated IgG position (2.6 mg) were combined and lyophilized. Half the sample was taken up into 200 µl buffer (0.1 M phosphate *p*H 7.0, 0.01 M cysteine, 0.002 M EDTA) and treated with 15 µg papain at 37 °C for 16 h. In the subsequent Ultrogel chromatography designed to obtain Fab fragments, we observed no intact IgG.

mitogenic when aggregated by antibody[9]. However, for these larger molecules, it is possible that the antisera have multiple specificity; the simpler octapeptide used here allows clearer definition of the effects.

Since our initial report of the conversion of a pure antagonist to agonist[10], we have shown that a different antagonist when converted to a dimer, could be activated using anti-serum[11], thus confirming the phenomenon. Here, use of purified immunoglobulin and peptide monomer allows firm conclusions to be made concerning the nature of the hormone-receptor interactions.

The high specificity of the hormone for the receptor presumably involves several amino acids, and the antigenic determinants of proteins and polypeptides have also been shown to encompass several amino acids[12]. Thus, it is unlikely that residues contained in the antigen-binding site are available for receptor binding at the same time and vice versa; the logical conclusion is that the octapeptide cannot bind more than one antibody molecule concurrent with the receptor site being occupied by this inhibitor. The IgG preparation, which is a divalent protein specific to the peptide N-terminus, can produce a biological response from an inactive hormone complex. This indicates that formation of an occupied receptor dimer (and no greater aggregation is required) by lateral movement through the membrane is a necessary step in the response pathway after binding of hormone to the receptor. Accepting that the octapeptide is only large enough to bind to the receptor and to one antibody molecule, it follows that the C-terminal region must have a dominant role in binding to the receptor, and this is common to both agonist and antagonist. That the anti-antagonist antibody recognizes the N-terminal region is supported by the fact that the anti-agonist antibody (Table 1) was unable to induce LH release, thus it is proposed that the ⌊Glu-His-Trp-Ser sequence is important in the dimerization of occupied receptors. We conclude, therefore, that this small peptide hormone is able to exert an effect on a normal cell population by having a sequence allowing specific binding, followed by a second sequence that causes dimerisation of the receptors, thus leading to biological expression by an as yet undefined series of stages.

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Tumour promotion by TCDD in skin of HRS/J hairless mice

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) serves as the prototype for a group of environmental toxicants, the halogenated aromatic hydrocarbons[1–3], which evoke a characteristic pattern of acute toxic and biochemical responses, and which produce these effects by stereospecific and reversible binding to a soluble protein receptor[2,4,5]. The chronic administration of TCDD[6–9] (and several other halogenated aromatic hydrocarbons)[10–13] to rats and mice has produced an increased incidence of hepatocellular carcinomas as well as tumours in other tissues. However, because TCDD is not a mutagen[14,15] and does not bind appreciably to DNA *in vivo*[16], it has been suggested that this compound is not a complete carcinogen, but rather acts as a tumour promoter, enhancing neoplastic expression in already initiated cells[17,18]. TCDD has been shown to be a potent tumour promoter in a two-stage model of carcinogenesis in rat liver[19], but has proven ineffective in the classical model in mouse skin (using CD-1 and Swiss-Webster mice)[20,21]. We have recently shown that TCDD produces epidermal hyperplasia and hyperkeratosis, and squamous metaplasia of the sebaceous glands in mice bearing the recessive trait, *hairless* (*hr*), but causes no changes in the skin of mice which are wild type or heterozygous at this locus[22]. Here we examine the capacity of TCDD to promote tumour formation in the skin of HRS/J hairless (*hr/hr*) mice and their congenic, haired (*hr*/+) littermates. Following the application of an initiating dose of a carcinogen, repeated skin application of TCDD produced papillomas in *hr/hr* mice but not *hr*/+ mice. In HRS/J hairless mice, tumour promotion by TCDD elicits the same incidence and multiplicity of skin papillomas as does the classical tumour promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), but at 1/100th the dose.

HRS/J is an inbred strain of mice segregating for the *hr* locus[23]; haired mice (*hr*/+) and hairless mice (*hr/hr*) are genetically identical except at the *hr* locus and closely linked loci on chromosome 14. Initiation of the skin of HRS/J (*hr*/+) haired mice with dimethylbenz(*a*)anthracene (DMBA 0.2 μmol) and subsequent twice-weekly topical application of the classical tumour promoter TPA, 2 μg per mouse, produced skin papillomas (Fig. 1*a*). Administration of DMBA alone or repeated application of TPA without prior initiation produced no tumours. Compared with other mouse strains[24], HRS/J haired mice are relatively sensitive to this regimen of DMBA/TPA: by 11 weeks of promotion, 100% of the mice developed papillomas, and by 14 weeks the tumour multiplicity (number of papillomas/number of surviving mice) was 9.2 papillomas per mouse. Following initiation with DMBA, repeated topical administration of TCDD (20 ng per mouse twice weekly for 8 weeks, then 50 ng per mouse twice weekly) failed to produce any papillomas over the 25-week course of the experiment.

Table 1 Comparison of TCDD and TPA as tumour promoters in HRS/J hairless mice

			At 20 weeks		
Initiation	Promotion		Tumour incidence (surviving mice with tumours/surviving mice)		Tumour multiplicity (average no. of papillomas/surviving mice)
MNNG	Acetone		1/19	5%	0.05
MNNG	TCDD	3.75 ng*	11/20	55%	0.7
MNNG	TCDD	7.5 ng	13/17	77%	1.5
MNNG	TCDD	15 ng	10/10	100%	4.0
MNNG	TCDD	30 ng	15/19	79%	1.6
Acetone	TCDD	30 ng	0/19	0%	0
MNNG	TPA	1 μg	5/19	26%	0.4
MNNG	TPA	3 μg	13/18	72%	1.6

HRS/J hairless female mice, 7 weeks old, were administered a single dose of MNNG (5 μmol in 50 μl of acetone) or the solvent alone applied to the dorsal skin, and then treated twice weekly with various doses of TCDD or TPA (in 50 μl of acetone) for 20 weeks. There were initially 20 mice per group and the survival was 85% or more in all groups except group 4 (TCDD 15 ng). Ten animals died in this group after 15 weeks of promotion, from dehydration coincident with being changed to a new type of cage. These deaths did not alter the tumour incidence or tumour multiplicity, that is, at 15 compared with 16 weeks.

* Dose of compound per mouse, given twice weekly.

In contrast, HRS/J (*hr/hr*) hairless mice, initiated with DMBA and treated with the same regimen of TCDD, did develop papillomas (Fig. 1*b*). After 25 weeks of TCDD

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promotion, 15 of 19 surviving mice (79%) had one or more skin tumours, with an average multiplicity of 1.4 tumours per mouse. Administration of DMBA alone produced a single papilloma, and in the absence of initiation, TCDD produced no papillomas. The time course of tumour incidence and tumour multiplicity produced by promotion with TCDD (20 ng per mouse, twice weekly) and TPA (2 μg per mouse, twice weekly) were very similar (Fig. 1*c*). Thus, TPA promotes tumour formation in the skin of both HRS/J haired and hairless mice, while TCDD is effective only in *hr*/*hr* mice.

A further comparison of TPA and TCDD is shown in Table 1. HRS/J hairless mice were initiated with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) and treated twice weekly with various doses of TCDD or TPA. TCDD produced a dose-related increase in the incidence and multiplicity of skin papillomas at doses of 3.75, 7.5 and 15 ng per mouse. At the highest dose tested, 30 ng per mouse, TCDD produced greater toxicity and was less effective as a tumour promoter. Comparable tumour promotion was produced by twice-weekly administration of TCDD at 7.5–15 ng per mouse ($2.3\text{–}4.6 \times 10^{-11}$ mol) and TPA at 3 μg per mouse (4.8×10^{-9} mol); thus, TCDD seems to be ~100 times more potent than TPA on a molar basis. However, one cannot formally compare the relative potencies of these compounds unless they act by a similar mechanism and produce the same maximum response with parallel log dose–response curves.

We examined the capacity of several halogenated aromatic hydrocarbon congeners and commercial mixtures of halobiphenyl isomers to promote tumour formation in the skin of HRS/J hairless mice (Table 2). 2,3,7,8-Tetrachlorodibenzofuran (TCDBF) and 3,4,5,3′,4′,5′-hexabromobiphenyl, both approximate isostereomers of TCDD which bind to the cytosol receptor and evoke a number of receptor-mediated responses including induction of microsomal monooxygenase activity[5,25], terminal differentiation of XB cells in culture[26] and hyperplasia and hyperkeratosis in the skin of hairless mice[22], are effective tumour promoters. TCDBF (1 μg per mouse, twice weekly) produced a greater tumour multiplicity than a near-optimally effective regimen of TCDD, and the TCDBF-treated mice showed less toxicity and better weight gain. The relatively rapid metabolism of TCDBF in the mouse[27] may permit attainment of a high local skin concentration of the compound without systemic accumulation and toxicity. 2,7-Dichlorodibenzo-*p*-dioxin and 2,4,5,2′,4′,5′-hexabromobiphenyl, congeners which neither bind to the cytosol receptor nor produce the characteristic biochemical and toxic effects of TCDD[22,25], were inactive as tumour promoters at the doses tested. Aroclor 1254 (1 mg per mouse twice weekly), a commercial mixture of chlorinated biphenyl isomers which has been widely used in industry in capacitors and transformers, as a lubricant and heat transfer fluid, slightly enhanced the incidence of skin tumours in MNNG-initiated mice, but this effect was not statistically significant. In contrast, Firemaster FF-1, a commercial mixture of brominated biphenyl isomers used as a flame retardant[28], was an effective tumour promoter, but the dose regimen used was toxic and produced severe hepatomegaly and hepatic porphyria.

Several aspects of these experiments require further comment. First, the epidermal and systemic effects produced by repeated skin painting with TPA and TCDD are in sharp contrast. In HRS/J haired and hairless mice, TPA produces an acute inflammation and hyperplasia of the epidermis, with little systemic toxicity. TCDD produces no gross or histological changes in the skin of *hr*/+ mice[22]. Hairless mice, repeatedly treated with TCDD, develop a scaly, shiny and taut appearance to their skin, and histologically one observes epidermal hyperplasia and hyperkeratosis, squamous metaplasia of the sebaceous glands, and accumulation of keratinaceous material in the dermal cysts which are normally found in *hr*/*hr* mice. In contrast to TPA, TCDD produces no acute inflammatory response in the skin, neither oedema nor leukocyte infiltration[22]. TCDD and other halogenated aromatic hydrocarbons produce several dose-related systemic lesions in both *hr*/*hr* and *hr*/+ mice: reduced weight gain or, at high doses, weight loss, loss of cutaneous and abdominal fat, thymic atrophy and liver enlargement and parenchymal cell changes[29]. Hairless mice repeatedly administered high doses of TCDD have skin which is easily damaged and may develop abscesses about the face and in the preputial and rectal glands. The animals assume a humped stature, with the back arched above the shoulders, and they move about poorly.

Second, the incidence and multiplicity of tumours produced by initiation and TPA promotion are less in HRS/J hairless mice than in haired mice, as noted previously by Giovanella *et al.*[30] and seen in Fig. 1*b*, *c*. Thus, the relatively low tumour multiplicity produced by TCDD and congeners in HRS/J *hr*/*hr* mice is a constraint of the animal model, and not a reflection of a poor promoting efficacy of these compounds.

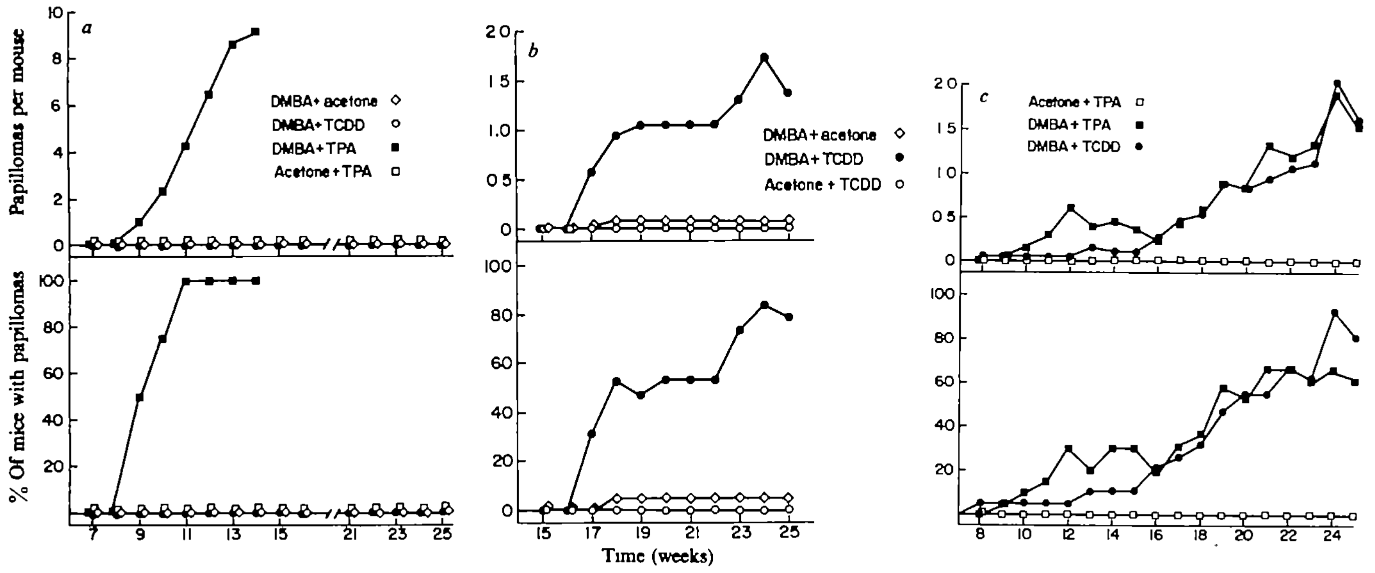

Fig. 1 Tumour promotion by TPA and TCDD in the skin of HRS/J *hr*/+ and *hr*/*hr* mice. *a*, Haired (*hr*/+) mice were shaven, initiated with a single dose of DMBA (0.2 μmol) applied to the skin, or with solvent (acetone), and then painted twice weekly with TPA (2 μg per mouse), TCDD (50 ng per mouse for 8 weeks, then 20 ng per mouse) or acetone. *b*, Hairless (*hr*/*hr*) mice: treatment regimens same as in *a*. *c*, Hairless (*hr*/*hr*) mice were administered DMBA (0.2 μmol) twice, 1 week apart, or the solvent, and then topically treated twice weekly with TPA (2 μg per mouse) or TCDD (20 ng per mouse). For experiments in *a*–*c*, each treatment group consisted of 20 female mice, and had 16 or more survivors at the end of the experiment. All compounds were dissolved in acetone. for initiation 100 μl of the acetone solution of DMBA was applied to *hr*/+ and *hr*/*hr* mice, and for promotion 50 μl of acetone or acetone solutions were applied to *hr*/*hr* mice, and 100 μl of these solutions to *hr*/+ mice.

Table 2 Tumour promotion by halogenated aromatic hydrocarbons in HRS/J hairless mice

Group	Initiation	Promotion	Dose (dose per mouse, twice weekly)	*n*	20 weeks Tumour incidence (surviving mice with tumours/surviving mice)		20 weeks Tumour multiplicity (average no. of papillomas/surviving mice)
1	MNNG	Acetone		26	0/23	0%	0
2	Acetone	TCDD	50 ng, 5 weeks, then 20 ng	26	0/18	0%	0
3	MNNG	TCDD	50 ng, 5 weeks, then 20 ng	26	16/19	84%	1.6
4	MNNG	2,3,7,8-Tetrachlorodibenzofuran	1.0 μg	20	19/19	100%	4.9
5	Acetone	2,3,7,8-Tetrachlorodibenzofuran	1.0 μg	20	1/20	5%	0.05
6	MNNG	3,4,5,3′,4′,5′-Hexabromobiphenyl	20 μg	20	12/20	60%	1.5
7	Acetone	3,4,5,3′,4′,5′-Hexabromobiphenyl	20 μg	20	0/20	0%	0
8	MNNG	2,4,5,2′,4′,5′-Hexabromobiphenyl	20 μg	20	0/20	0%	0
9	Acetone	2,4,5,2′,4′,5′-Hexabromobiphenyl	20 μg	26	0/22	0%	0
10	MNNG	2,7-Dichlorodibenzo-*p*-dioxin	20 μg	20	0/19	0%	0
11	Acetone	2,7-Dichlorodibenzo-*p*-dioxin		20	0/20	0%	0
12	MNNG	Aroclor 1254	1 mg	20	4/19	21%	0 3
13	Acetone	Aroclor 1254	1 mg	20	0/19	0%	0
14	MNNG	Firemaster FF-1	2 mg, 5 weeks, then 1 mg	26	9/15	60%	2.0
15	Acetone	Firemaster FF-1	2 mg, 5 weeks, then 1 mg	20	1/16	6%	0.13

HRS/J hairless female mice, 8 weeks old, received a single administration of MNNG (5 μmol in 50 μl of acetone) or solvent alone applied to the skin, and were then treated topically twice weekly with test compound dissolved in 50 μl of acetone for 20 weeks Treatment with Firemaster FF-1 produced a significant mortality and the dose was reduced after 5 weeks The appreciable mortality in other groups (2, 3 and 6) occurred largely in the first 6 weeks of the experiment, resulted primarily from dehydration and did not seem to be a result of the treatment regimen. Additional animals were added to these groups, so all groups consisted of 20 or 26 mice.

Third, skin tumours elicited by promotion with TCDD (and congeners) were often smaller (1–3 mm) and progressed more slowly than those elicited by promotion with TPA. Virtually all the tumours were keratinizing squamous cell papillomas, regardless of the promoter used. Finally, in all treatment groups (Fig. 1*a–c*), the survival rate was >80%. HRS/J mice harbour a murine leukaemia virus, and the expression of the recombinant polytropic virus and the incidence of lymphatic leukaemia are greater in *hr/hr* mice than *hr/+* mice[31–33]. By 10 months of age, leukaemia develops in 45% of *hr/hr* mice and only 1% of the heterozygote *hr/+* mice[31]. In all experiments with *hr/hr* mice (Fig. 1*b*, *c*, Tables 1, 2), 0–15% of the animals developed leukaemia (as evidenced by enlarged spleen, thymus, lymph nodes and/or liver) which either resulted in their premature death or was detected at autopsy at the termination of the experiment. The incidence of leukaemia was not significantly different in any of the treatment groups, and seemed to be independent of exposure of the animals to a carcinogen or halogenated aromatic hydrocarbons.

TCDD and related halogenated aromatic hydrocarbons comprise a class of potent tumour promoters which seem to act by a mechanism that is distinct from that of TPA and its congeners. The epidermal hyperplasia[22] and most probably the tumour promotion (as suggested by the structure–activity relationship in Table 2) produced in hairless mice by these halogenated aromatic hydrocarbons are mediated through the cytosol receptor. In contrast, the promoting activities of TPA, other phorbol and diterpene esters, and structurally unrelated compounds such as teleociden and debromoaplysiatoxin[34–37], apparently act through another mechanisms.

Two aspects of this model of tumour promotion in the skin of hairless mice may prove especially useful. First, comparison of the pleiotropic responses produced by TCDD in the skin of congeneic HRS/J *hr/hr* and *hr/+* mice may permit identification of the additional battery of genes expressed in *hr/hr* skin which are in some manner associated with promotion. Second, it may be determined if any of the biochemical effects produced by TPA and congeners and implicated in their mechanism of action[18] are also common to the response produced by halogenated aromatic hydrocarbons.

Our model of tumour promotion by TCDD and congeners may be relevant to humans because the hyperplastic/metaplastic response produced in the skin of the mutant hairless mouse is similar to that evoked by these compounds in human skin.

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Surface sialic acid reduces attachment of metastatic tumour cells to collagen type IV and fibronectin

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Attachment of cells to substrate containing fibronectin, laminin and collagens has been shown to influence cell proliferation[1], differentiation[2,3] and migration[4], aspects of cellular behaviour known to be altered by malignant transformation. Recent studies on mutant cell lines displaying altered attachment to substrate and changes in cell-surface carbohydrate composition have provided evidence for the involvement of glycoconjugates in cell attachment[5–7]. Using this approach, we have now found that a non-metastatic wheat germ agglutinin-resistant (WGA^R) mutant of the highly metastatic murine tumour MDAY-D2 has a three- to fourfold reduction in neuraminidase-accessible cell-surface sialic acid which seems to allow enhanced attachment to basement membrane collagen type IV and fibronectin, but not to laminin. Furthermore, neuraminidase treatment of the WGA-sensitive metastatic tumour cells enhanced their attachment to collagen type IV and fibronectin, but not to laminin. Neuraminidase treatment had no effect on attachment of the WGA^R mutant cells to any of the substrates.

Yogeeswaran and Salk[8] have recently shown that the metastatic capacity of a range of murine tumour cell lines is positively correlated with the degree of sialation of available galactose and *N*-acetylgalactosamine residues on cell-surface glycoconjugates. This correlation is supported by the finding that some WGA^R mutants of the B16 melanoma are non-metastatic[9] and deficient in cell-surface sialic acid[10]. WGA binds preferentially to the oligosaccharide portions of glycoconjugates containing *N*-acetylglucosamine and sialic acid[11]. The lectin must bind to the cell surface to exert its toxic effects and, as might be expected, some genotypic classes of WGA^R cells have less surface sialic acid and hence show decreased WGA binding[12]. MDW40, the non-metastatic WGA^R mutant of MDAY-D2, also binds less WGA[13,14] and shows a three- to fourfold reduction in neuraminidase-accessible sialic acid compared with the parental tumour cells and two WGA-sensitive (WGA^S) revertants designated W40M1 and W40M4 (Table 1). The highly metastatic WGA^S revertants are lines established from metastatic nodules found in the organs of mice injected with the WGA^R mutant. MDW40 cells have previously been shown to grow only at the subcutaneous site of injection where over time, one or a few cells become WGA sensitive and highly metastatic. The revertant tumour cells progressively dominate the primary tumour cell population and metastasize to distant organs[14,15] (for example, W40M1 and W40M4). The origin and phenotypes of the tumour cell lines are summarized in Table 1. These observations are similar to those of Bramwell and Harris who have shown that some WGA^R tumour cell variants which are poorly tumorigenic revert at the site of inoculation to the WGA-sensitive and more malignant phenotype[16].

Two WGA^R mutants of MDAY-D2 have previously been shown to be highly immunogenic and for this reason, poorly tumourogenic in the syngeneic host. In contrast MDW40 is highly tumourogenic, poorly immunogenic and completely non-metastatic, differing from the parental tumour line in only the latter phenotype[17]. Interaction between tumour cells and the host which do not involve immune stimulation are known to influence metastatic capacity. In particular, metastatic tumour cells must invade local tissues, move into and out of the circulation and grow at secondary sites, processes which by definition require contact between the tumour cell and host extracellular matrix proteins.

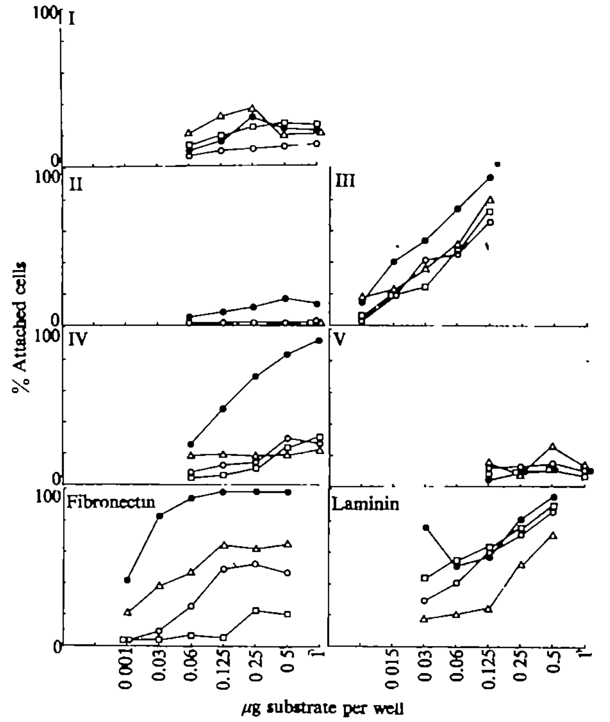

Fig. 1 Adhesion of untreated tumour cells to purified collagens, laminin and fibronectin. Replicate wells in Terasaki Microtest plates (Falcon Plastics) were coated with the following substrates; collagen types I (lathyritic rat skin), II (bovine articular cartilage), III (fetal bovine skin), IV (mouse EHS tumour) and V (human placenta), laminin (mouse EHS sarcoma) and fibronectin (human plasma). The purification of these proteins followed standard procedures[23–27] Each well of the Microtest plates was coated with 5 μg bovine serum albumin (BSA) and concentrations of substrate ranging from 0.001 to 1 μg. The BSA and substrates were allowed to dry for 2 h Tumour cells from tissue culture in log growth phase were washed three times in PBS at 4 °C, adjusted to 5×10^4 per ml in Dulbecco's minimal essential medium (DMEM)/F12 nutrient mixture (3/1, v/v), 15 mM HEPES, *p*H 7, and 10-μl aliquots of the cell suspensions were added to triplicate wells, incubated at 37 °C for 30 min then turned upside-down and incubated for a further 30 min. Cells adhering to the substrate, and those falling to the base of the droplet were counted and the mean per cent adhering calculated from triplicate counts. Background adhesion to wells coated with BSA alone ranged from 0 to 10%. The data represent the mean of three experiments. ●, MDW40; △, MDAY-D2; ○, MDW40M1; □, MDW40M4.

The non-metastatic WGA^R mutant, the parental tumour line and two metastatic revertant lines were compared for their ability to attach to laminin, fibronectin and collagen types I–V using a modification of a sensitive drop assay[18]. Compared with the metastatic lines, attachment of MDW40 to collagen type IV and fibronectin was increased (Fig. 1). A small increase in MDW40 attachment to collagen type III was also apparent. Tumour cell attachment to collagens I, II and V was minimal and no differences between the various lines were observed. The four tumour cell lines readily attached to laminin but there were no consistent differences between attachment of the mutant and wild-type cells to this substrate.

The tumour cells were grown in suspension cultures and showed a rounded morphology and negligible attachment to the Falcon tissue culture plastic. With one exception the cells displayed the same rounded morphology following attachment to each of the substrates. MDW40 cells spread only on fibronectin at concentrations >0.03 μg per well and required 2–3 h for optimal flattening. Although MDW40 cells attached to fibronectin at concentrations of <0.03 μg per well they did not flatten, which is consistent with the interpretation that cells require a minimal number of attachment points to spread[19]. The weak attachment of the metastatic cell lines to fibronectin

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may therefore preclude spreading. MDW40 attached firmly to plastic coated with extracellular matrix produced by endothelial cells in tissue culture. After 3 days of growth on this substrate a tightly packed layer of polygonal shaped cells was formed. The metastatic cell lines remained rounded and loosely attached to plastic coated with endothelial cell extracellular matrix (data not shown). It is known that growth of some tumour cell lines on endothelial cell extracellular matrix results in a more differentiated cell morphology, reflected by firm attachment, cell spreading and formation of non-overlapping cell monolayers[3].

In an attempt to mimic the attachment phenotype of the mutant, tumour cells were pretreated with neuraminidase and compared with sham-treated cells for their ability to adhere to laminin, fibronectin and collagen types III and IV (Fig. 2). Neuraminidase treatment had no effect on adhesion of MDW40 cells but greatly enhanced that of MDAY-D2, W40M1 and W40M4 cells to collagen type IV and fibronectin. Similar though less marked effects were observed with collagen type III as substrate, and no differences were observed with laminin. The results indicate that decreased cell-surface sialic acid can increase cell attachment to fibronectin and collagen type IV while not affecting attachment to laminin. Therefore, cell attachment to fibronectin and collagens may be mediated by cell-surface receptors different from those which mediate attachment to laminin. Selective inhibition of hepatocyte attachment to laminin or fibronectin by the same respective proteins in soluble form has led to identical conclusions[20].

Our results indicate that a mutation leading to increased cell-surface sialic acid may produce a less adhesive cell. Furthermore, some ricin-resistant mutants of baby hamster kidney cells have been shown to bind less ricin due to masking of the ricin receptors by sialic acid and to be less adhesive to fibronectin-coated plastic than the parental cell line[6,7]. These results and our own are consistent with the proposal that cell attachment to certain substrates such as fibronectin and collagen type IV requires carbohydrate chains which are potential acceptors of terminal sialic acid residues.

Table 1 Comparison of neuraminidase-accessible sialic acid on WGA^{R} mutant and wild-type cells

Tumour cell lines	Phenotype*	Release of counts from ^{3}H-N-acetyl mannosamine-labelled cells (%)†	Thiobarbituric acid assay (nM sialic acid per mg cell protein)‡
MDAY-D2	Met, WGA^{S}, Oua^{S}	16.7 ± 5.1	$1\ 91 \pm 0.35$ (5)
MDW40	Non Met, WGA^{R}, Oua^{R}	$4\ 1 \pm 1.8$	0.59 ± 0.13 (4)
W40M1	Met, WGA^{S}, Oua^{R}	22.2 ± 9.5	1.89 ± 0.06 (2)
W40M4	Met, WGA^{S}, Oua^{R}	$16\ 0 \pm 5\ 7$	1.69 ± 0.12 (3)

* The origin of the highly metastatic (Met) DBA/2 tumour MDAY-D2 has been described in detail previously[24]. WGA (2–5 $\mu g\ ml^{-1}$) and ouabain (0.1 mM) added to MDAY-D2 in tissue culture reduced cell growth by 90% (D_{10}) (WGA^{S}, Oua^{S}). MDW40 is a ouabain-resistant (Oua^{R}) mutant ($D_{10} = 3$ mM) of MDW4, a non-metastatic WGA^{R} mutant ($D_{10} = 50\ \mu g\ ml^{-1}$) of MDAY-D2[14]. MDW40 cells have been shown to grow only locally at the site of a subcutaneous inoculation and when 10^{5} cells are injected intravenously less than 5% of the mice die of tumour takes. Recent experiments have demonstrated that 28–35 days after intravenous injection of MDW40 cells, one or a few tumour cells form hybrids with a host cell *in situ*, acquiring the WGA-sensitive phenotype[15]. The WGA-sensitive tumour cells selectively dominate the subcutaneous tumour cell population and metastasize to distant organs, resulting in death 60–90 days after tumour cell inoculation. W40M1 and W40M4 are WGA^{S}, Oua^{R} resistant tumour cell lines isolated from the liver and spleen respectively of two DBA/2 mice injected subcutaneously with 10^{5} MDW40 cells 90 days earlier.

† Tumour cells, 5×10^{5} per ml, were cultured in RPMI plus 10% fetal calf serum and 2 $\mu Ci\ ml^{-1}$ [6-^{3}H]*N*-acetylmannosamine. After 48 h, the cells were washed three times in Dulbecco's phosphate-buffered saline (PBS) and samples of 10^{6} cells in 100 µl of PBS, 5 mM $CaCl_2$, *p*H 6.1, were incubated alone (*C*) or with 0.02 U of neuraminidase (Behringwerke) (*N*) for 60 min. at 37°C. The cells were washed twice in PBS, solubilized and radioactivity was measured The formula $(C - N/C) \times 100$ was used to calculate the percentage of neuraminidase-accessible sialic acid[10] The results are the mean ± s.e. of three experiments.

‡ Tumour cells were washed three times and 20×10^{6} cells in 0 5 ml PBS, 5 mM $CaCl_2$, *p*H 6.1, were incubated with 2 U of neuraminidase (Sigma, type V) for 60 min at 37°C. The cells were pelleted and sialic acid in the supernatant was measured using the thiobarbituric acid assay Protein was measured using the Bio-Rad reagent and ovalbumin as the standard. The results represent the mean ± s.e. of the number of experiments indicated in parentheses.

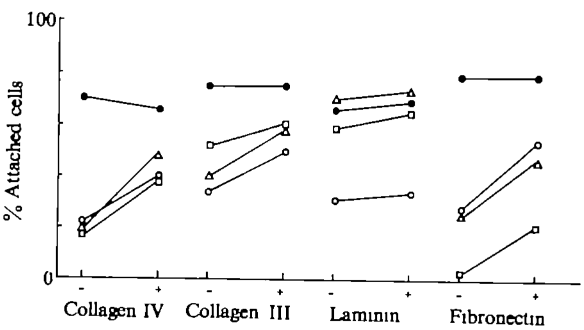

Fig. 2 The effect of neuraminidase treatment on adhesion to collagens, laminin and fibronectin Cells were washed three times in PBS, and two samples of 10^{6} cells in 200 µl of Dulbecco's PBS, *p*H 6.9, were incubated with and without 0.02 U of neuraminidase from *Arthrobacter woafociens* (Behringwerke) at 37 °C for 30 min. 2 ml of PBS was then added, the cells were centrifuged, the supernatant was removed and a further treatment with 0.02 U neuraminidase carried out The cells were then washed three times in cold PBS, resuspended at 5×10^{4} ml in DMEM/F12 nutrient mixture (3/1, v/v), 15 mM HEPES, *p*H 7, and kept on ice The adhesion of treated and untreated cells to fibronectin (0 03 µg per well), laminin (0 25 µg per well) collagen III (0.06 µg per well) and collagen IV (0.25 µg per well) was measured. The data represent the mean of three experiments. Neuraminidase treatment enhanced binding of the metastatic cell lines to collagen IV ($P < 0.05$), fibronectin ($P < 0.05$) and collagen III ($P < 0.1$), but not to laminin ($P < 0\ 95$) as determined by a paired *t*-test. ●, MDW40; △, MDAY-D2; ○, MDW40M1; □, MDW40M4.

The specific glycoconjugates involved in cell attachment have not been clearly defined. Glycolipids terminating in galactose have been shown to mediate homotypic adhesion[21] and polysialylated gangliosides seem to inhibit fibronectin-mediated cell attachment in a nonspecific manner[22]. These findings are consistent with our results and with the hypothesis that specific glycoconjugates terminating in galactose (that is, non-sialylated *N*-linked complex or *O*-linked mucin type as well as glycolipids) may be receptors for a lectin-like interaction with fibronectin.

In a manner analogous to ricin binding, sialation of the oligosaccharide may reduce fibronectin binding. These results do not preclude the earlier suggestion that sialic acid increases the net negative charge of the cell and effects a charge repulsion of the cell and substratum[23]. However, this is less likely since changes in sialation did not effect cell attachment to laminin.

The avidity of attachment to substrate may change cellular behaviour. Compared with their normal counterpart, transformed cells are known to be less adhesive and less dependent on substratum for growth, properties which may allow the cells to escape from the primary tumour. Firm attachment of cells can induce differentiation[2] and contact inhibition of growth[1]. Our results suggest that the increase in sialation of the metastatic tumour cell surface[8] can result in decreased attachment to basement membrane proteins collagen type IV and fibronectin, predisposing the tumour cells to increased mobility and decreased growth control by substratum contact.

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Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour

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The neuroregulation of growth hormone (GH) secretion is mediated in part by a stimulatory, GH-releasing factor (GRF) and an inhibitory peptide, somatostatin[1], both of which reach the adenohypophysis by the hypothalamic-hypophysial portal system[2]. Somatostatin has been shown to be a tetradecapeptide[3,4], a large form of which was later also sequenced[5]. The existence of a GRF is supported by physiological evidence[6–9] and by reports of GH-releasing activity in hypothalamic extracts[10–17]. Although several agents with GH-releasing activity have been identified[14–16], none is of high potency and fulfils the criteria expected of a physiological GRF[18,19]. GH-releasing activity observed in extracts of carcinoid and pancreatic islet tumours removed from patients with GH hypersecretion, acromegaly and pituitary adenoma or hyperplasia has been partially characterized[20–23]. We report here the sequence of a 40 residue GH-releasing peptide purified from a pancreatic islet tumour described earlier by Thorner *et al.*[23]. A synthetic replicate of this peptide, termed human pancreatic tumour GRF [hpGRF(1–40)]-OH, co-elutes on HPLC with the native peptide and is highly potent in stimulating GH secretion *in vitro* and *in vivo*. A close structural homology with the peptides of the glucagon–secretin family, and more particularly with PHI[24], is recognized. Structure–activity studies show that hpGRF(1–29)-NH$_2$ has full intrinsic activity and potency *in vitro*.

hpGRF was purified from a pancreatic tumour removed from a subject who presented symptoms and signs of acromegaly and increased GH levels that rapidly abated after surgical removal of the tumour[23]. Throughout the purification, we used an *in vitro* method for assaying the ability of a putative GRF to stimulate the secretion of GH by primary cultures of rat pituitary cells (see ref. 25 and Fig. 3 legend).

The tumour was processed in two batches (I and II) of 2.5 and 5 g respectively. Batch I allowed us to optimize extraction and chromatographic conditions that led to the final two-step purification of hpGRF from batch II (see Fig. 1 legend for details). Approximately 6 nmol of >80% pure GRF were obtained, corresponding to an overall recovery of >50%.

Both purification steps yielded one major zone of GRF-like activity. The independent processing of side cuts from the gel permeation or generated during reverse-phase HPLC purification indicated that very closely related molecules having GRF activity may be present in the extracts. Their relative amounts, however, could not be more than 5% of the total on the basis of absorbance readings. Furthermore, it is not excluded that these distinct active entities may have been the result of molecular alterations (oxidation of methionine or partial hydrolysis) that occurred during processing of the fractions.

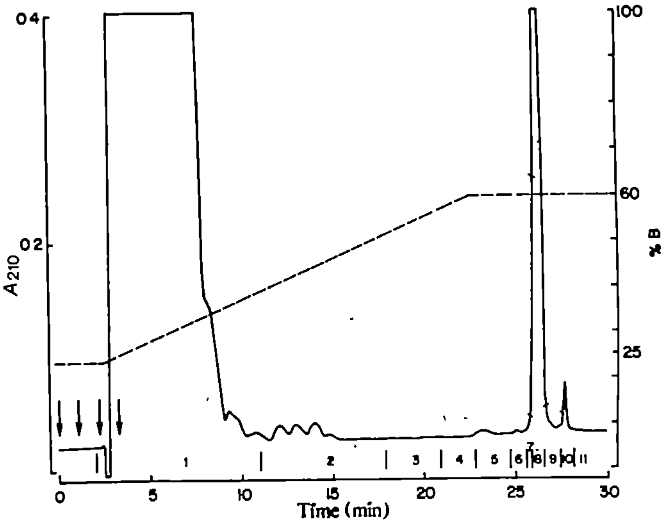

Fig. 1 Extraction and gel permeation chromatography of hpGRF· batch II was lyophilized, polytroned in 10 vols acetone, maintained at −20 °C for 2 days and centrifuged. The acetone precipitate was extracted in hot (>90 °C) acid (1 M acetic acid + 0.1 M HCl) containing 10 mM EDTA and 0.5% β-mercaptoethanol (β-ME) and 5 µg ml^{-1} pepstatin, heated for 2 min, polytroned, chilled and centrifuged. The supernatant was defatted by 3×4 vols ether/petroleum ether (1·2) and applied to a 3.5×120 cm column containing Sephadex G-50 fine and eluted with 3 M acetic acid/0.5% β-ME at a flow rate of 20 ml h^{-1} Fractions (9.4 ml) were collected, aliquots were lyophilized and assayed in pools. GH-releasing activity was detected in fractions 55–76 corresponding to K_{av} 0.20–0.38. HPLC purification of hpGRF: the zone containing most of the GRF activity (K_{av} 0.24–0.33) was applied through multiple injections (4×1 ml) to a Vydac diphenyl end-capped (5 µm, 300 Å pore size, 4.5×250 mm) column (three equivalent runs were performed with the following loads 1/5 and twice 2/5 of total), which was then eluted with an acetonitrile gradient (3→30% in 25 min) in TEAP buffer[37]. The activity was associated with several absorbance peaks eluting 22–25 min after the start of the gradient (flow rate 1 2 ml min^{-1}). A second HPLC step was performed on the pool of the active fractions from the previous run after partial evaporation of the acetonitrile in a spin vacuum concentrator. A Vydac C$_{18}$ (5 µm, 300 Å, 4.5×250 mm) column was eluted with an acetonitrile gradient (21→36% in 20 min) at a flow rate of 1.2 ml min^{-1} after multiple injections (2×1 ml) of the concentrated fractions at the relative loads described earlier. All the activity was associated with one symmetrical absorbance peak eluting 21 5 min after the start of the gradient. The total pool of the active fraction (injection volume of 3.5 ml loaded in three aliquots of 1 ml and one of 0.5 ml) was re-run on the same Vydac C$_{18}$ end-capped column at a flow rate of 1.2 ml min^{-1} and room temperature in the gradient conditions shown in the figure (A = 0.1% trifluoroacetic acid (TFA) in water, B = 0.1% TFA in 60% CH$_3$CN/40% water) to concentrate hpGRF and free it, before sequencing, from β-ME which had been added to each tube at each step in order to retain a high level of biological activity. Indeed, partial oxidation of methionine-27 to methione sulphoxide leads to a significant loss of activity (~2% of that of the parent compound) as was shown with synthetic analogues. Fraction 8 was subsequently submitted to composition and sequence analysis Native hpGRF and hpGRF(1–40)-OH co-elute on HPLC under conditions (Vydac C$_{18}$, 1.2 ml min^{-1}, 27→31% CH$_3$CN in 20 min) that would separate them from all other analogues described here including hpGRF(1–40)-NH$_2$.

The primary structure of purified hpGRF was determined by Edman degradation in a Beckman 890C spinning cup sequencer, modified according to Wittmann-Liebold[26], to be: Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-. The sequencing strategy and results are described elsewhere[27] and summarized in Fig. 2 legend.

Fully protected hpGRF(1–40)-NH$_2$ was synthesized in a stepwise manner on a p-methylbenzhydrylamine resin and hpGRF(1–40)-OH was synthesized on a chloromethylated resin

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according to the general solid-phase approach of Merrifield[28] using experimental procedures described earlier[29,30]. After complete deprotection and cleavage by hydrofluoric acid, the crude preparations of hpGRF(1–40) and analogues were purified using preparative HPLC techniques developed in our laboratory (see Fig. 3 legend). The abilities of native hpGRF, synthetic hpGRF(1–40)-OH, hpGRF(1–40)-NH_2, and 8-bromo cyclic AMP to stimulate GH secretion by cultured rat pituitary cells are compared in Fig. 3. Both synthetic peptides and the native hpGRF exhibit similar potencies and intrinsic activities in this assay. In multiple culture assays, the minimally effective concentrations of synthetic hpGRF(1–40)-OH are 3–8 pM, the EC_{50} values 20–100 pM and the maximally effective concentrations are reached by 1 nM. Thus the *in vitro* potency of the synthetic peptide is either similar to or greater than the potencies of the hypophysiotropic peptides isolated from the hypothalamus (thyrotropin-releasing hormone, gonadotropin-releasing hormone, somatostatin[19] and corticotropin-releasing factor[31]).

We have synthesized several extended and shortened analogues of hpGRF(1–40) and tested them *in vitro*. The following peptides exhibited similar (within a factor of 2) potencies to hpGRF(1–40)-OH *in vitro*: hpGRF(1–29)-NH_2, hpGRF(1–32)-NH_2, hpGRF(1–39)-NH_2, hpGRF(1–40)-NH_2, hpGRF(1–40)-Phe-NH_2, hpGRF(1–40)-Phe-OH and hpGRF(1–40)-Phe-Gln-NH_2. These results demonstrate that the N-terminal 29 residues of hpGRF possess all the information required for full *in vitro* activity and suggest that the C-terminal region of hpGRF is not critical for receptor recognition. Full intrinsic activity is observed in hpGRF(1–27)-NH_2 which possesses 10–20% of the potency of hpGRF(1–40)-OH.

hpGRF shows striking structural homology with the peptides of the glucagon family, particularly its new member PHI[24] (Fig. 2). hpGRF(1–27) and PHI have 12 common residues in an equivalent position, while 12 additional residues could represent single base changes. We have not observed any GH-releasing activity of PHI, (Gln[24])-PHI, vasoactive intestinal peptide or glucagon on anterior pituitary cells. However, the activity of 27- and 29-residue hpGRF analogues suggests that hpGRF may be further processed biologically with maintenance of hypophysiotropic activity.

It is not known whether hpGRF is normally produced in pancreatic islets or if this is an ectopic source for the peptide, or what is the relationship between the putative hypothalamic GRF and hpGRF-(1–40). Natural hpGRF and partially purified rat hypothalamic GRF, which exhibit similar chromatographic behaviour on Sephadex G-50, can be separated by HPLC. However, other observations suggest that they are structurally similar. There is ample precedence for a close structural relationship between peptides produced ectopically by tumours and those produced normally[32]. Furthermore, partial sequence analysis of the N-terminal region of highly purified rat hypothalamic GRF showed sequence homology with hpGRF (J.S., J.R. and W.V. unpublished results).

Biologically, hpGRF(1–40) and closely related peptides exhibit many properties expected of a putative GH-releasing factor[33]. Thus, synthetic hpGRF and purified rat hypothalamic GRF fractions exhibit parallel dose–response curves and intrinsic activities *in vitro* (Fig. 4). In agreement with our studies[13] using purified rat hypothalamic GRF, and others[17,34] using purified hypothalamic or pancreatic tumour GRF, we find that somatostatin non-competitively blocks GH release due to synthetic hpGRF[33]. However, even at the highest somatostatin concentrations, hpGRF can still elicit some GH secretion. As with native tumour GRF[34], synthetic hpGRF-mediated GH secretion is calcium-dependent[33]. In agreement with Cronin *et al.*[35] who used native hpGRF, we find that synthetic hpGRF increases intracellular cyclic AMP and that somatostatin attenuates this rise (L. Bilezikjian and W.V., in preparation). The GH-releasing activity of synthetic and native hpGRF is strongly potentiated by pretreatment of cells with glucocorticoids and thyroid hormone[33]. hpGRF is also highly potent *in vivo*. A

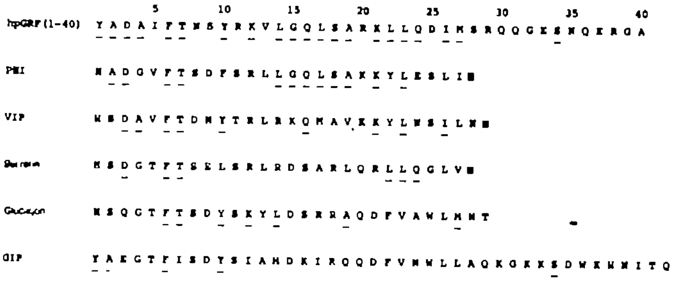
5 10 15 20 25 30 35 40
hpGRF(1-40) Y A D A I F T N S Y R K V L G Q L S A R K L L Q D I M S R Q Q G E S N Q E R G A
PHI H A D G V F T S D F S K L L G Q L S A K K Y L E S L I ■
VIP H S D A V F T D N Y T R L R K Q M A V K K Y L N S I L N ■
Secretin H S D G T F T S E L S R L R D S A R L Q R L L Q G L V ■
Glucagon H S Q G T F T S D Y S K Y L D S R R A Q D F V A W L M N T
GIP Y A E G T F I S D Y S I A M D K I R Q Q D F V N W L L A Q K G K K S D W K H N I T Q

Fig. 2 Comparison of the amino acid sequences of hpGRF and other members of the glucagon–secretin family. Residues of a given structure that are identical with the corresponding one of hpGRF are underlined. The primary structure of hpGRF was determined by Edman degradation. The phenylthiohydantoin derivatives of the amino acids were identified by reverse-phase HPLC[38] Several sequencing experiments, each using 0.7–1.2 nmol of peptide, were performed. The peptide was applied directly to the sequencer cup with or without previous treatment with 3-sulphophenylisothiocyanate or after incubation with trypsin, staphylococcal protease or BrCN that cleaved the peptide at different sites. The mixture of fragments was sequenced without purification The proposed primary structure hpGRF(1–40)-OH shown above satisfies sequence[27] and C-terminal (tritiation) analyses[40], and HPLC data. Amino acid analyses performed using previously reported methodology[39] gave the following composition as integers· 5 Asx, 2 Thr, 4 Ser, 7 Glx, 1 Pro, 3 Gly, 4 Ala, 2 Val, 1 Met, 2 Ile, 5 Leu, 2 Tyr, 2 Phe, 3 Lys, 4 Arg. Extra residues are likely to be associated with contaminating peptide(s). Gel filtration and finger printing data[27] exclude the possibility that hpGRF is significantly larger. VIP, vasoactive intestinal peptide; GIP, gastric inhibitory polypeptide

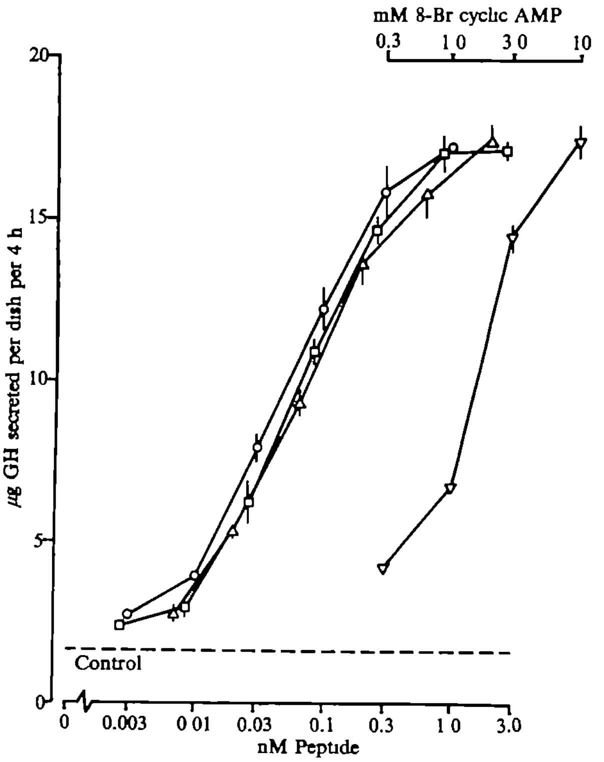

Fig. 3 Effects of native hpGRF (○), synthetic hpGRF(1–40)-NH_2 (□), synthetic hpGRF(1–40)-OH (△) and 8-bromo cyclic AMP (▽) on GH secretion over a 4 h period by rat pituitary cells in monolayer culture for 3–5 days. Cells were dissociated and cultured as described in refs 25, 33. Culture medium for this and other assays reported here include 5 nM dexamethasone and 30 pM triiodothyronine. GH levels are estimated by radioimmunoassay using NIADDK National Hormone and Pituitary Program kits prepared by Dr A. Parlow. For purification of the synthetic peptides, cartridges fitting Waters Associates prep LC-500 were packed with 17 μm C_{18} silica (300 Å; Vydac). A gradient of CH_3CN in TEAP[37] was generated by a low-pressure Eldex gradient maker. Desalting of the purified fractions independently checked for purity was achieved using a gradient of CH_3CN in 0.1% TFA. The centre cut was then lyophilized to yield the desired 40-residue peptide, the purity of which can be >98% (J.R., in preparation).

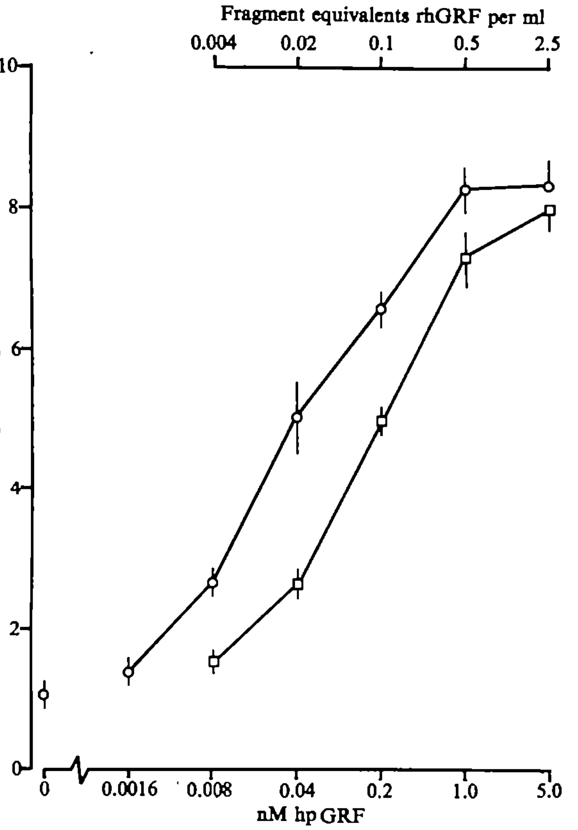

Fig. 4 Effects of synthetic hpGRF (○) and purified rat hypothalamic GRF (rhGRF; □) on secretion of GH by cultured rat pituitary cells. rhGRF was purified as described in ref. 33.

dose-related increase in GH plasma levels was observed (C. Rivier, J.R., J.S. and W.V., in preparation) after administration of from 40 ng per kg to 25 μg per kg of synthetic hpGRF to freely running catheterized rats pretreated with FLA-63, a dopamine β-hydroxylase inhibitor that suppresses spontaneous GH secretion[36].

The association of elevated GH levels in the patient with an hpGRF-containing tumour suggests that synthetic hpGRF and its analogues may be powerful GH secretagogues in humans and be of use in the diagnosis and treatment of human disorders.

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Identification of plasmid (pKM101)-coded proteins involved in mutagenesis and UV resistance

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Mutagenesis of *Escherichia coli* by UV and many chemicals is not a passive process and requires the intervention of host functions. The isolation of chromosomal *umuC* mutants, which are nonmutable by many DNA-damaging agents and are slightly sensitive to them, has led to the model that the *umuC* gene product(s) functions in a pathway of 'error-prone repair'[1-3]. *UmuC* mutants are suppressed by the introduction of the plasmid pKM101[4]; pKM101 was derived from the clinically isolated plasmid R46 by *in vivo* manipulations[5]. The *muc* (*m*utagenesis, *U*V and *c*hemical) region of pKM101 is required for this suppression and we have suggested that it is an analogue of the chromosomal *umuC* gene(s)[6]. We have now subcloned the *muc* region onto the high-copy number vector pKB354 and identified two protein products by gel electrophoresis of maxicell preparations. Complementation analysis of *muc* insertion mutations has confirmed the existence of two genes, *mucA* and *mucB*, whose protein products are required for pKM101's effects on mutagenesis and resistance to killing. The molecular weights (MWs) of the *mucA* and *mucB* proteins are 16,000 and 45,000 respectively.

The plasmid pKM101 has played an important part in the Ames test for detecting carcinogens as mutagens[7,8]. pKM101 confers on its host enhanced capacities for survival and mutagenesis that are $recA^+lexA^+$ dependent[9] and apparently inseparable by Tn*5* insertion mutagenesis[6]. Such insertions identified an approximately 1.6 kilobase (kb) region of pKM101 necessary for these phenotypes which we termed *muc*.

To determine whether the *muc* region is also sufficient for the expression of these phenotypes, we have now subcloned it into the multiple-copy number vector pKB354 (Ap^R, Tc^R; K. Backman, unpublished). pKB354 is a derivative of pBR322 which has lost the *Sal*I/*Hin*cII site in the *tet* gene, yet remains tet^+. *Hpa*I fragments of the pKM101 derivative pGW270 (ref. 10) were subcloned into the *Hin*cII site in the *bla* gene of

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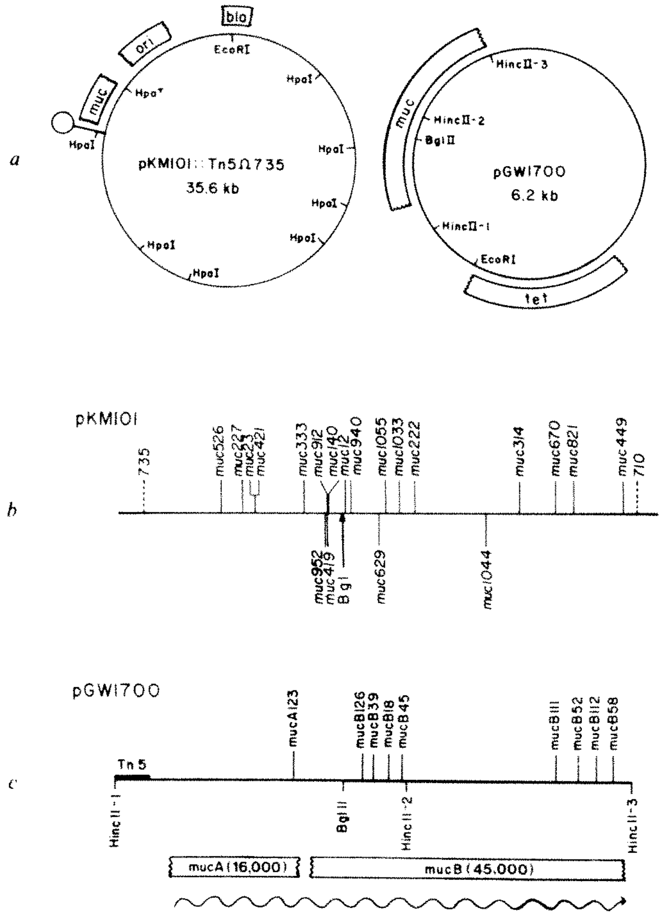

Fig. 1 *a*, Partial restriction map of pKM101::Tn*5Ω735* and pGW1700 showing the relative orientation of the *muc* region of the two plasmids. The construction of pGW1700 involved the insertion of a *Hpa*I fragment containing *mucA*, *mucB* and the end of Tn*5Ω735* into the *Hinc*II site of the high-copy number vector pKB354. *b*, Expanded map of the pKM101 *muc* region showing the location of Tn*5* insertions affecting mutagenesis. pKM101Ω710 and pKM101Ω735 are Tn*5* insertions which flank the *muc* region. *c*, Expanded map of the pGW1700 *muc* region showing the location of Tn*1000* insertions affecting mutagenesis and the position and direction of transcription of *mucA* and *mucB* (molecular weights in parentheses).

pKB354 and transformed into TK610 (*umuC36*, *uvrA6*[1]). Colonies were selected for tetracycline resistance and screened for ampicillin sensitivity and UV mutability. The screen for UV mutability made use of the revertible *his-4* marker of TK610. Tc^RAp^S colonies were spread as patches on minimal media containing a trace amount of histidine (0.02 mM) and irradiated with 2 J m^{-2} of UV from a germicidal lamp. Muc^+ patches were easily identified as they gave rise to as many as 100 His^+ revertants whereas the control patches rarely had more than one His^+ revertant.

In this manner, several Muc^+ derivatives were identified that contained hybrid plasmids in which the *muc* region was inserted into pKB354 in either of the two possible orientations. The expression of *muc* was qualitatively similar regardless of its orientation. One derivative, pGW1700, was selected for further studies. pGW1700 mediates the reactivation of UV-irradiated λ phage (Fig. 3*a*) and suppresses the nonmutability of *umuC36* strains by methyl methanesulphonate (MMS; Fig. 4) to a greater extent than does pKM101. These results indicate that the *muc* region is indeed sufficient for the expression of plasmid-mediated enhancement of survival and mutagenesis.

The proteins encoded by pGW1700 were examined on SDS-polyacrylamide gels using maxicell preparations[11] labelled with ^{35}S-methionine. The maxicell procedure takes advantage of the fact that, in *recA* cells, irradiation with appropriate doses of UV leads to the degradation of chromosomal but not plasmid DNA, thus allowing plasmid-coded proteins to be labelled specifically. pGW1700 encodes two proteins of MWs 16,000 and 45,000 respectively, which are not encoded by the vector (Fig. 2, tracks 1, 2). To determine whether these proteins are products of the *muc* gene(s), we isolated *muc* insertion mutants of pGW1700 generated by the transposable element Tn*1000* (ref. 12) and looked for truncated peptides. Initial attempts to obtain pGW1700 *muc*::Tn*1000* insertions used GW2100 (*umuC*::Tn*5*) as the recipient and proved difficult for reasons that are still unknown. Introduction of pGW2, a pkm101 *muc* mutant derived by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine treatment[13], into GW2100 facilitated the isolation of such mutants. *Muc* insertions isolated in this manner map, by restriction endonuclease analysis, between the *Bgl*II and *Hinc*II-3 sites of pGW1700. Figure 1 compares the sites of the Tn*1000* insertions with those of the *muc*::Tn*5* insertions previously isolated in pKM101.

Maxicell preparations of the pGW1700 mutants *mucB58*::Tn*1000*, *mucB112*::Tn*1000* and *mucB126*::Tn*1000* lack the 45,000 MW protein, which we have identified as the product of the *mucB* gene (Fig. 2, tracks 3–5). Truncated peptides, resulting from translation termination occurring within Tn*1000* sequences[11], are present in the *mucB58*::Tn*1000* and *mucB112*::Tn*1000* extracts. Their sizes suggest that the direction of transcription of *mucB* is towards the *Hinc*II-3 site of pGW1700. (The ends of *mucB* are defined by the *mucB58*::Tn*1000* insertion and by a fusion of the 5' end of the gene to *lacZ* at the *Bgl*II site; S. J. Elledge and G. C. W., unpublished results.) None of the initial pGW1700 *muc*

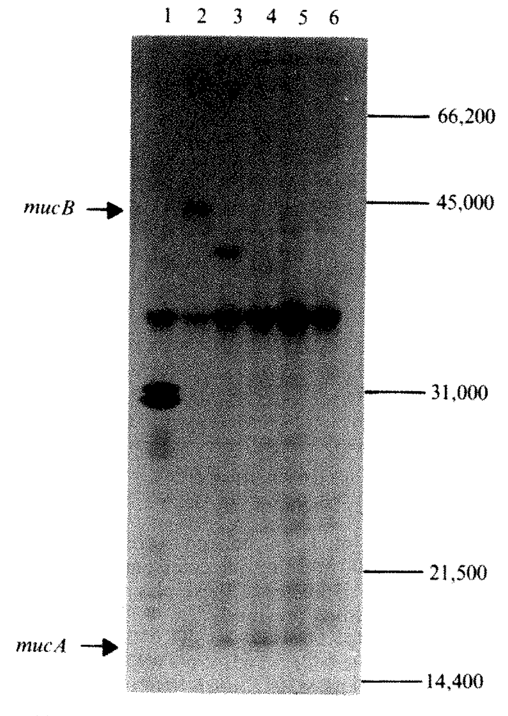

Fig. 2 ^{35}S-methionine-labelled proteins produced in maxicells by the plasmids pKB354 and pGW1700 (*mucA*$^+$, *mucB*$^+$) and by derivatives of pGW1700 in which Tn*1000* is inserted in either the *mucA* gene or the *mucB* gene. Maxicell extracts were prepared from plasmid-containing derivatives of RB901 (*spr*, *recA*) following UV irradiation to 35 J m^{-2}. Proteins were separated by electrophoresis on a 10–18% gradient polyacrylamide gel and visualized by fluorography. Arrows indicate the positions of the *mucA* and *mucB* proteins. Tracks 1, pKB354; 2, pGW1700; 3, pGW1700 *mucB58*::Tn*1000*; 4, pGW1700 *mucB112*::Tn*1000*; 5, pGW1700 *mucB126*::Tn*1000*; 6, pGW1700 *mucA123*::Tn*1000*. Molecular weights of the *mucA* and *mucB* proteins were determined by reference to unlabelled protein standards following staining with Coomassie blue. Molecular weights of the standards are indicated on the side.

insertion mutations affected the synthesis of the 16,000-MW protein. We suspected that such mutations were complemented by pGW2 in the GW2100 recipient and therefore went undetected. The use of GW2100(pKM101 *mucA421*::Tn*5*)[6] as a recipient permitted the isolation of pGW1700 *mucA123*::Tn*1000*. *mucA123*::Tn*1000* mutants fail to synthesize both the 45,000-MW *mucB* protein and the 16,000-MW *mucA* protein in maxicell preparations (Fig. 2, track 6). The apparent polarity of the *mucA123*::Tn*1000* mutation suggests that *mucA* and *mucB* constitute an operon, the *mucA123*::Tn*1000* insertion being in the promoter-proximal *mucA* gene.

The compatibility between pKM101 and pGW1700 derivatives has enabled us to carry out complementation analyses of several *muc* alleles which have revealed that both the *mucA* and *mucB* gene products are required for plasmid-mediated mutagenesis and repair. A deletion derivative of pKM101 *mucA421*::Tn*5* was constructed which lacks the internal *Xho*I fragments[14] of Tn*5*. Such deletions are reported to relieve polarity effects caused by insertion of the transposon[14]. *MucA421*::Tn*5*Δ*Xho*I and *mucB112*::Tn*1000* alleles complement to a moderate extent when tested for the capacity to reactivate UV-irradiated λ phage (Fig. 3*b*). The original insertion mutation, *mucA421*::Tn*5*, also complements *mucB112*::Tn*1000*, but to a lesser degree. Thus it seems that the *mucA421*::Tn*5* insertion is incompletely polar on *mucB*. Complementation of phage reactivation between *mucB1044*::Tn*5* and *mucA123*::Tn*1000* is also observed, but at a very low, yet reproducible, level (Fig. 3*a*).

Similar results were obtained in complementation analyses of MMS mutability, determined by reversion of the ochre *argE3* marker (Fig. 4). *mucA421*::Tn*5* and *mucB112*::Tn*1000* complement to mediate MMS mutagenesis to a level equivalent to that mediated by pKM101. Again, the *mucB1044*::Tn*5* and *mucA123*::Tn*1000* alleles complement to a lesser degree. We suspect that the differences in the extent of complementation reflect differences in the degree of polarity caused by the *mucA421*::Tn*5* and *mucA123*::Tn*1000* insertions. Alternatively, complementation may be affected by gene dosage effects caused by copy number differences between pKM101 and pGW1700 derivatives. No complementation of MMS mutability or phage reactivation was observed between *mucA421*::Tn*5* and *mucA123*::Tn*1000* or between *mucB1044*::Tn*5* and *mucB112*::Tn*1000*.

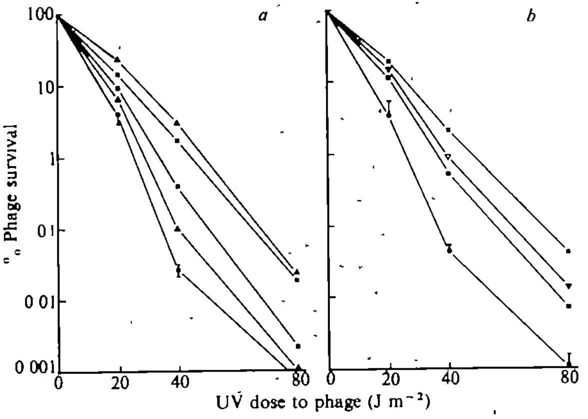

Fig. 3 Reactivation of UV-irradiated λcI857 mediated by pKM101 and pGW1700 and showing complementation between several *mucA* and *mucB* alleles of those plasmids. Reactivation was measured as described previously[6]. *a*, ●, TK610 (*umuC36*, *uvrA6*); ■, TK610(pKM101); ▲, TK610(pGW1700); □, TK610(pKM101 *mucA421*::Tn*5*, pGW1700 *mucB112*::Tn*1000*); △, TK610(pKM101 *mucB1044*::Tn*5*, pGW1700 *mucA123*::Tn*1000*). The following strains, also assayed for λcI857 reactivation, gave results similar to those of TK610: TK610(pKM101 Tn*5*), TK610(pGW1700 *mucA123*::Tn*1000*), TK610(pGW1700 *mucB112*::Tn*1000*), TK610-(pKM101 *mucA421*::Tn*5*, pGW1700 *mucA123*::Tn*1000*), TK610(pKM101 *mucB1044*::Tn*5*, pGW1700 *mucB112*::Tn*1000*). The range of values for these strains is indicated by the bars. *b*, ●, TK610(pKM101 *mucA421*::Tn*5*); ■, TK610(pKM101); ∇, TK610(pKM101 *mucA421*::Tn*5*Δ*Xho*I, pGW1700 *mucB112*::Tn*1000*); □, TK610 (pKM101 *mucA421*::Tn*5*, pGW1700 *mucB112*::Tn*1000*). The following strains, also assayed for λcI857 reactivation, gave results similar to those of TK610(pKM101 *mucA421*::Tn*5*): TK610(pKM101 *mucA421*::Tn*5*Δ*Xho*I) and TK610(pGW1700 *mucB112*::Tn*1000*). The range of values for these strains is indicated by the bars.

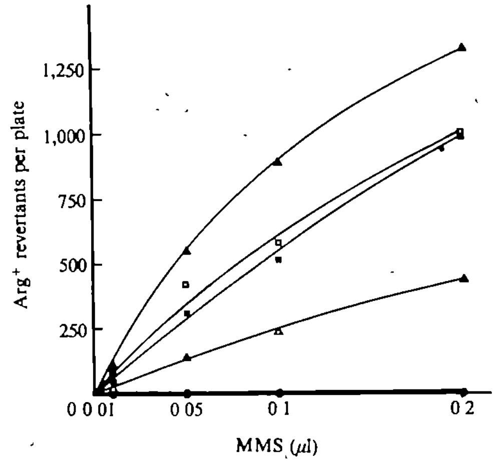

Fig. 4 MMS-induced reversion of the *argE3* mutation mediated by pKM101 and pGW1700 and showing complementation between several *mucA* and *mucB* alleles of those plasmids. To 2 ml of top agar supplemented with limiting (0.05 mM) arginine and nonlimiting concentrations of all other growth requirements were added 0.1 ml of a fresh stationary phase culture and the appropriate amount of a freshly prepared solution of MMS in dimethyl sulphoxide. The top agar was then poured on minimal-glucose plates which were then incubated at 37 °C for 4 days. Spontaneous revertants have been subtracted. ●, GW3201(TK610 *argE3*, *zii2*::Tn*5*); ■, GW3201(pKM101); ▲, GW3201(pGW1700); □, GW3201(pKM101 *mucA421*::Tn*5*, pGW1700 *mucB112*::Tn*1000*); △, GW3201(pKM101 *mucB1044*::Tn*5*, pGW1700 *mucA123*::Tn*1000*). The following strains, also included in the assay, were indistinguishable from GW3201: GW3201 (pKM101 *mucA421*::Tn*5*), GW3201(pKM101 *mucB1044*::Tn*5*), GW3201(pGW1700 *mucA123*::Tn*1000*), GW3201 (pGW1700 *mucB112*::Tn*1000*), GW3201(pKM101 *mucA421*::Tn*5*, pGW1700 *mucA123*::Tn*1000*) and GW3201(pKM101 *mucB1044*::Tn*5*, pGW1700 *mucB112*::Tn*1000*).

These results indicate that both *mucA* and *mucB* gene products are required for mutagenesis and repair, and that the phenotype of *mucA* insertions is not due simply to their polar effects on *mucB*. As we have yet to discern any phenotypic differences between *mucA* and *mucB* mutations, it seems likely that the two gene products act in concert to mediate mutagenesis and repair.

The chromosomal *umuC* locus of *E. coli* has recently been cloned and found to consist of two genes, *umuD* and *umuC*, that code for gene products of MWs 16,000 and 45,000 respectively[15]. The pKM101 *mucA* and *mucB* gene products are thus very similar in size to the chromosomally encoded *umuD* and *umuC* gene products, further supporting the hypothesis[4] that the proteins of the *muc* and *umuC* loci have analogous roles in the processing of damaged DNA that gives rise to mutations. We are now investigating the possibility of complementation between *muc* and *umu* alleles. As no *muc* alleles that we tested complemented the *umuC36* mutation carried by the host, either the individual *muc* gene products are insufficiently homologous to complement separately the corresponding *umu* gene products or the *umuC36* mutant is defective in both *umuD* and *umuC*. The identification of the *mucA* and *mucB* proteins should facilitate a systematic analysis of the biochemistry of error-prone repair.

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A transposon in *Streptococcus faecalis* with fertility properties

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Certain strains of streptococci have been shown to be capable of conjugatively transferring drug resistance determinants in the apparent absence of plasmid DNA[1–6]. The transferrable tetracycline resistance (Tc^r) marker of *Streptococcus faecalis* DS16 has been located on a transposon, designated Tn *916*[2,4,7]. We show here that when chromosomal Tn *916* is conjugatively transferred it inserts into a number of different sites on the recipient chromosome. Our studies also imply that transfer and transposition share a common step, which may be excision of the transposon from the donor chromosome.

The ability of Tn*916* to transfer from plasmid-free donors (see Table 1 for description of bacterial strains) in overnight filter matings is shown in the data of Table 2. DS16C3 is able to transfer Tc^r at a frequency of about 10^{-8} to the plasmid-free recipient strain JH2-2. Transconjugants such as CG130 are capable of transferring Tc^r to the isogenic recipient JH2SS at a similar frequency. To investigate the nature of this transfer event, chromosomal DNA isolated from the donor and transconjugant strains listed in Table 2 was digested with *Hin*dIII restriction endonuclease, resolved by agarose gel electrophoresis, and transferred to nitrocellulose paper using the Southern technique[8]. The transferred DNA was probed with a ^{32}P-labelled *Eco*R1 restriction fragment (F') of pAM211 (a derivative of pAD1, a resident plasmid of DS16) that contains the entire transposon. There are no *Eco*R1 sites within Tn*916* and about 85% of the F' fragment consists of Tn*916* sequences. As Tn*916* contains a single *Hin*dIII site, two chromosome–transposon junction fragments should be resolvable after hybridization with the probe. The resulting autoradiogram (Fig. 1*a*) shows that three different transconjugants obtained from the DS16C3×JH2-2 mating, detailed in Table 2, display hybridization patterns (lanes 2–4) that differ from those seen in the donor (lane 1) as well as from each other. One such transconjugant, CG140, gave rise to two chromosome–transposon junction fragments (X and Y), whereas two other transconjugants, CG110 and CG130, gave rise to four or more bands. Figure 1*a* also shows the hybridization profiles of transconjugants derived from the secondary mating described in Table 2 that had used strain CG130 as the donor. Again the hybridization bands of the transconjugants (lanes (5–7) differed from those of the donor as well as from each other. Strain CG132 gave rise to a multiple band pattern, whereas two other transconjugant strains, CG131 and CG133, exhibited the simpler banding pattern.

The variation in hybridization profiles amongst different transconjugants implies that Tn*916* inserts at different sites on the recipient chromosome. These findings also argue against the possibility that Tn*916* existed on a plasmid which had escaped physical detection. If the latter had been the case, identical patterns should have been observed in the transconjugants.

The patterns of multiple bands observed with transconjugants CG110, CG130, and CG132 were reproducible, and do not represent incomplete digestion products. An autoradiogram of a Southern blot hybridization experiment involving *Eco*R1 digested chromosomal DNA and ^{32}P-labelled Tn*916* probe is shown in Fig. 1*b*. As expected, a single hybridizing fragment was detected in the *Eco*R1 digested chromosomal DNA from strains CG133, CG131, and DS16C3 (strains that gave rise to two hybridizing fragments in the *Hin*dIII digestion experiments—see Fig. 1*a*). By contrast, those transconjugants that gave rise to complex band patterns following *Hin*dIII digestion (CG132, CG130, and CG110) gave rise to more than one hybridizing fragment when digested with *Eco*R1. In every case, the hybridizing fragments following *Eco*R1 digestion of chromosomal DNA were larger than ~15 kilobase. These data strongly suggest the presence of non-tandem multiple copies of the entire transposon in strains CG110, CG130, and CG132; however, the possibility that major rearrangements of the transposon give rise to these data cannot be completely eliminated. We note that restriction enzyme analyses of several pAD1::Tn*916* plasmids formed by transposition from the CG110 chromosome revealed a typical ~15 kilobase insertion (data not shown).

Tc^r transconjugants of JH2-2 that exhibit altered transfer frequencies in secondary matings were readily obtainable, and usually represented about 20% of the original transconjugants. Strain CG110 was capable of donating Tn*916* at frequencies of about 10^{-6} (Table 2). To investigate the transposition of Tn*916* in such a strain the conjugative haemolysin plasmid pAD1 was introduced into strains CG110 and CG130, and the frequency of Tn*916* transposition from the chromosome to pAD1 was measured by determining the number of Tc^r, hyper-haemolytic transconjugants obtained after filter-mating of these

Table 1 *S. faecalis* strains and relevant properties

Strain	Chromosomal genotype	Plasmid content	Reference
DS16C3	*tet*	None	4
JH2-2	*rif fus*	None	21
JH2SS	*str spc*	None	22
CG110	*rif fus tet*	None	This paper
CG130	*rif fus tet*	None	This paper
OG1-RF (pAM211)	*rif fus*	pAM211(Tn*916* inserted into *Eco*R1 F fragment of pAD1)	4
CG180	*rif fus*	pAM180 (pAM81::Tn*916*)	This paper
CG190	*str spc*	pAM190(Tn*917* inserted into *Eco*R1 B fragment and Tn*916* into *Eco*R1 D fragment of pAD1)	This paper

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Table 2 Tn*916* transfer from plasmid-free donors

Donor strain	Recipient strain	Frequency of Tc^r transconjugants per recipients*	Representative transconjugants
DS16C3	JH2-2	1×10^{-8}	CG110 CG130 CG140
CG130	JH2SS	2×10^{-8}	CG131 CG132 CG133
CG110	JH2SS	4×10^{-6}	

* Filter matings were carried out as described in ref. 4, using Difco antibiotic medium no. 3 (AB3), and an initial ratio of one donor per ten recipients. Antibiotics were used in selective agar plates at the following concentrations: tetracycline, 10 μg ml^{-1}; rifampin, 25 μg ml^{-1}; fusidic acid, 25 μg ml^{-1}; and streptomycin, 1000 μg ml^{-1}. For reasons discussed in ref. 4, we generally express frequencies in terms of recipients (rather than donors) in cases where the values are very low. Frequency of spontaneous Tc^r mutations was less than 10^{-10}.

donor strains with JH2SS. The hyperhaemolytic phenotype results from an insertion within or near the haemolysin determinant of pAD1, and can be readily detected by an increased diameter of haemolysis zones on horse blood agar plates[4]. The frequency of hyperhaemolytic, Tc^r transconjugants was found to be more than 200 times higher when CG110 was the donor strain (4.9×10^{-6} per recipient) than when CG130 was the donor strain (1.8×10^{-8}). pAD1, *per se*, transferred equally well ($\sim10^{-2}$) from both hosts. Similar results were obtained with another independently isolated variant, CG100, which transferred Tn*916* at frequencies about 10 times higher than normal; transposition from the CG100 chromosome to pAD1 was similarly increased. These data indicate Tn*916* transfer and transposition may share a common step.

When located on a conjugative plasmid, Tn*916* was found to excise at relatively high frequency upon transfer to a recipient strain. pAM180 is a derivative of the ~26 kilobase erythromycin-resistance (Em^r) plasmid pAM81 with Tn*916* inserted into the *Eco*R1 fragment G. When donor strains containing pAM180 were filter-mated with JH2SS, a high degree of segregation of the Em^r and Tc^r determinants occurred, as can be seen in the data of Table 3. Although both the Em^r and Tc^r determinants transferred at similar frequencies, a significant proportion of erythromycin selected transconjugants (43–77%) were not resistant to tetracycline.

Of those transconjugants selected for tetracycline resistance, however, greater than 90% were also erythromycin resistant. Interestingly, in some of the erythromycin selected transconjugants that were tetracycline resistant Em^r and Tc^r were no longer linked, as implied by a great reduction in the transfer frequency of Tc^r, but not Em^r, in secondary matings. The loss of the Tc^r determinant from the plasmid possibly relates to a zygotic induction of one or more Tn*916*-related recombination enzymes.

To determine the structural integrity of pAM81 DNA sequences in the various types of transconjugant, DNA from representative plasmids was digested with *Hin*dIII and analysed by agarose gel electrophoresis (Fig. 2*a*). Plasmid DNA isolated from two Em^rTc^r transconjugants in which Em^r and Tc^r were no longer linked (CG184 and CG186) exhibit a restriction pattern identical to that seen for pAM81. (Note the two fragments of pAM180 that had contained Tn*916* sequences were lost, and pAM81 fragment G reappeared). It is presumed that Tn*916* transposed to the bacterial chromosome in these strains since they were tetracycline resistant and capable of transferring Tn*916* at very low frequency. Plasmid DNA from strain CG187 (Em^rTc^s) also gave rise to a restriction pattern identical to that of pAM81. In contrast, plasmid DNA from strain CG181 (Em^rTc^r), in which both resistance determinants remained linked, displayed a restriction pattern identical to that of the pAM180 donor. These data support the view that transposition involves an excision followed by insertion, but that insertion is not necessarily coupled to excision—in which case the excised element is lost.

Analogous results were obtained in matings that used as a donor strain CG190. This strain harbors a pAD1 derivative (pAM190) with two transposon insertions: Tn*917* (Em^r) and Tn*916* (see Table 1). The Tn*916* insertion gave rise to a nonhaemolytic phenotype, yet the strain displays a hyperhaemolytic phenotype on blood plates that contain tetracycline (10 μg ml^{-1}). This drug-influenced phenotype has been observed previously in certain pAD1::Tn*916* derivatives containing transposon insertions into or near the haemolysin determinant[9]. CG190 transferred Em^r at a frequency of $\sim2\times10^{-4}$ per donor to JH2-2 in overnight broth matings; and Tc^s derivatives (for example CG191) as well as Tc^r derivatives in which the Em^r and Tc^r determinants were no longer linked (CG194) were found among the transconjugants at a frequency of about 4%. Agarose gel electrophoresis of *Eco*R1 digested plasmid DNA from such transconjugants (Fig. 2*b*) revealed the presence of a fragment (*Eco*R1 D fragment) that had been missing in the original pAM190 DNA as a result of Tn*916* insertion. Most significant was the additional observation that transconjugants CG191, CG192, and CG194 all regained the haemolytic phenotype, implying that the excision of Tn*916* was precise.

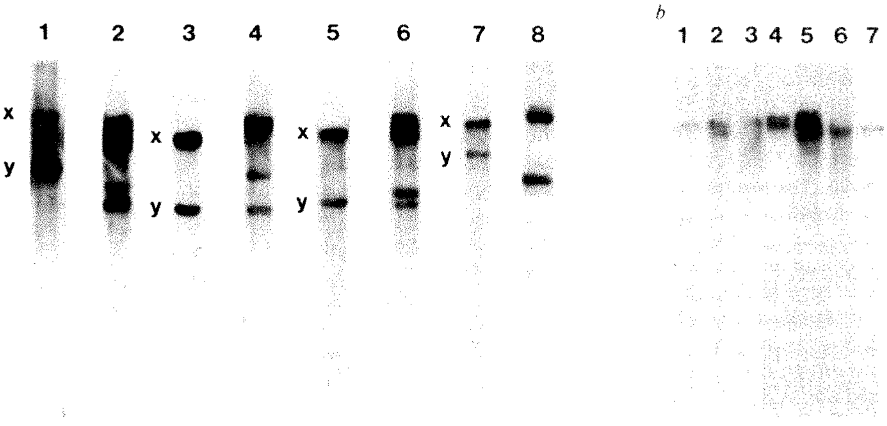

Fig. 1 Autoradiograms obtained from filter-blot hybridization analyses of chromosomal DNA from Tc^r transconjugants. *a*, *Hin*dIII digested DNA (1–2 μg) from transconjugants CG110, CG140, and CG130, (lanes 2–4 respectively) which were obtained using DS16C3 (lane 1) as donor. Lanes 5–7 contain *Hin*dIII digested DNA from transconjugants CG131, CG132, and CG133, respectively, which were obtained using CG130 as donor. Fragments marked x and y denote presumed chromosome–transposon junction fragments. Lane 8 contains *Hin*dIII digested pAM211 DNA and hybridizing bands are approximately 18.5 Kb and 7.5 kb. *b*, *Eco*R1-digested DNA (1–2 μg) from strains CG133, CG132, CG131, CG130, CG110, and DS16C3, (lanes 1–6 respectively). Lane 7 contains *Eco*R1 digested pAM211 DNA from which the probe had been made. Isolation of DNA by buoyant density gradient centrifugation and agarose gel electrophoresis was as described in ref. 4. DNA transfer and hybridization were essentially according to Southern[8] as modified by Wahl *et al.*[23], and used a BRL blot transfer system. The *Eco*R1 F′ fragment of pAM211 was purified by electroeluting the DNA from an agarose gel slice placed in a dialysis bag filled with electrophoresis buffer that had been embedded in 1% agarose. Following electrophoresis[4] the DNA was recovered by ethanol precipitation. Nick translation of pAM211 *Eco*RI F′ probe DNA with either ^{32}P-dCTP (670 Ci mmol^{-1}) or ^{32}P-dATP (410 Ci mmol^{-1}) was carried out using a nick-translation kit.

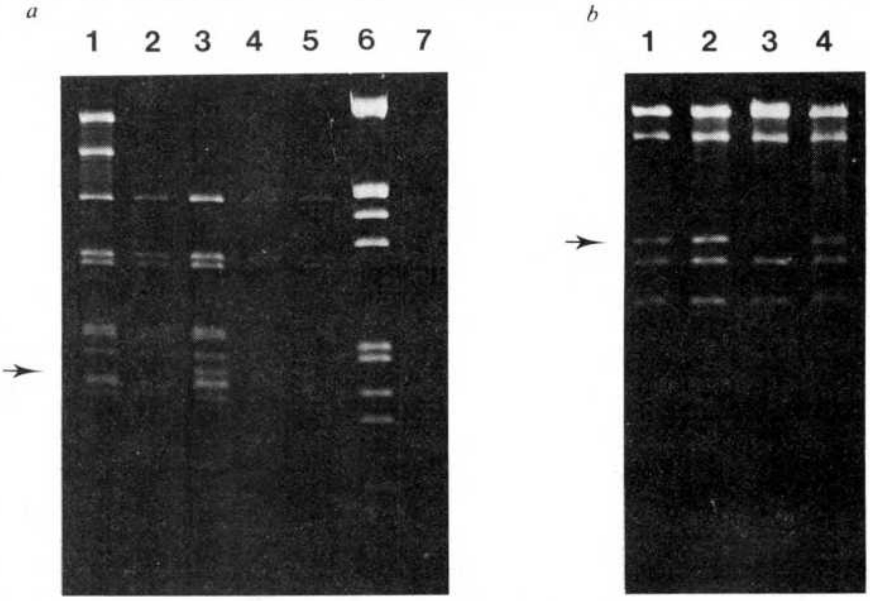

Fig. 2 Excision of Tn*916* from plasmid DNA after transfer to recipients. *a*, *Hin*dIII digests of transconjugant plasmid DNAs derived from the CG180 donor. Lanes 1 and 2 contain plasmid DNA from CG180 and CG130 (pAM81), respectively. The arrow marks fragment G, no longer present after Tn*916* insertion into pAM81. Lanes 3–5 contain plasmid DNA from the $Em^{r}Tc^{r}$ transconjugants CG184 and CG186, and the $Em^{r}Tc^{s}$ transconjugant CG187. Lane 7 contains plasmid DNA from the $Em^{r}Tc^{r}$ CG181 strain, and lane 6 contains λ DNA digested with *Eco*R1 and *Hin*dIII. *b*, *Eco*R1 digests of transconjugant plasmid DNAs derived from the CG190 donor. Lanes 1–4 contain plasmid DNA from the $Em^{r}Tc^{s}$ transconjugants CG191 and CG192, and the $Em^{r}Tc^{r}$ transconjugants CG193 and CG194. The restriction pattern obtained for pAM190 is identical to that of CG193 (data not shown). The arrow marks the *Eco*R1 fragment D of pAD1, lost as a result of Tn*916* insertion and regenerated after excision. The highest molecular weight band in lanes 1, 2 and 4 represents a doublet and includes the pAD1 *Eco*R1 A and B::Tn*917* fragments; whereas the highest molecular weight material in lane 3 represents a triplet which includes the *Eco*R1 D::Tn*916* fragment. (Broth matings were performed as in ref. 24.) Agarose gel electrophoresis utilized a 1% (Fig. 2*a*) or 0.7% (Fig. 2*b*) gel and Tris-borate buffer[25].

Transconjugants in which the Em^{r} and Tc^{r} markers remained linked, such as CG193, displayed an *Eco*R1 restriction pattern like the donor and were, as expected, nonhaemolytic.

On the basis of information known thus far we suggest the following model for Tn*916* behaviour. When located on the bacterial chromosome Tn*916* may spontaneously excise at low frequency. Although there is no direct evidence for the form the excised element might take, we think that it is most probably a circular molecule incapable of vegetative replication. Assuming that induction of key enzymes accompanies the excision step, the element now has the potential to: (1) insert back into the chromosome at a new, or perhaps the original site; (2) insert into a resident plasmid; (3) transfer into a recipient strain (if conditions warrant it); or (4) segregate. Conjugation could be viewed as occurring by a plasmid-like process, with zygotic induction of appropriate enzymes facilitating insertion into the recipient chromosome. The generation of multiple copies of Tn*916* in some transconjugants could arise by secondary transpositions (still via excision-insertion) from a replicated portion of the chromosome to an unreplicated region. Completion of replication thus results in one of the daughter chromosomes having two transposons. Conceivably when Tn*916* enters a new cell, a certain amount of time (a generation or so?) may be required to achieve levels of 'repressor' sufficient for stable maintenance of the element at one site. During this time there could be a tendency to move from one site to another.

Table 3 Segregation of Em^{r} and Tc^{r} in filter matings involving CG180 donors

	Em^{r} Tc^{r} transconjugants		
Experiment	Erythromycin selected*	Tetracycline selected	Erythromycin selected Em^{r} Tc^{r} transconjugants with unlinked markers
1	27/47 (57%)	41/43 (95%)	6/12
2	16/48 (33%)	46/48 (96%)	1/12
3	11/48 (23%)	44/48 (92%)	1/10

Tn*916* was inserted into pAM81 to generate pAM180 via overnight filter matings between CG110 (pAM81) and JH2SS followed by selection for tetracycline resistant transconjugants capable of transferring Tc^{r} linked to Em^{r} in secondary matings. (The plasmid pAM81, originally identified in *S. faecalis* DU81 is capable of conjugal transfer in filter matings at frequencies of about 10^{-4} per donor, Y. Yagi and L. Dempsey, personal communication.) Each donor was derived from a single colony isolate and was filter mated with JH2SS (JH2-2 in secondary matings) as described in Table 2. Frequency of Em^{r} and Tc^{r} transconjugants was 0.6–1.5×10^{-5} per donor.

* Although $Em^{r}Tc^{s}$ transconjugants appeared readily, random analyses of the CG180 donor failed to detect any Tc^{s} colonies (135/135 were $Em^{r}Tc^{r}$) implying dissociation of Tc^{r} occurred after, or during, transfer.

In view of the increasing reports of 'plasmid-free' transfer of resistance determinants, especially among the streptococci, Tn*916* may represent a prototype of a new class of genetic elements that could be referred to as 'conjugative transposons'. In this regard Smith and Guild[10] have recently obtained evidence suggesting that the conjugative multiple resistance element originating in *S. agalactiae* B109 may be located on a large transposon.

It is important to note that homology has recently been demonstrated between Tn*916* and the transferable Tc^{r} determinants (classified as *tetM*) of *S. pneumoniae* and *S. agalactiae*[11,12] as well as with a chromosomal Tc^{r} determinant (not yet shown to be transferable) in *S. mutans*[13]. Recent reports of transferable nonplasmid elements in *Clostridium difficile*[14] and *Bacteroides fragilis*[15–18] suggest that such elements may exist in a variety of prokaryotic genera (including Gram negative bacteria). Although certain R-plasmids in *Escherichia coli* (IncJ)[19] and *Haemophilis*[20] prefer to be integrated in the chromosome, their possession of replication functions and dependence on host recombination functions to integrate distinguishes these elements from Tn*916*.

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Regulatory sites for *his3* gene expression in yeast

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The expression levels of many genes are regulated in response to particular environmental or developmental cues. A simple regulated gene can conceptually be divided into three elements: structural components that encode the gene product, promoter elements that are essential for gene expression, and regulatory elements responsible for changing the level of expression after a specific stimulus[1,2]. When cells of the yeast *Saccharomyces cerevisiae* are subjected to amino acid starvation, *his3* and many other amino acid biosynthetic genes are expressed threefold above the basal level[3,4]. Previously, I isolated mutations mapping outside the structural gene that severely reduce or eliminate *his3* expression[5,6]. These mutations define two distinct *his3* promoter elements located 115–155 and 32–52 base pairs (bp) from the site of transcriptional initiation. Here I describe 18 small deletion mutations, some of which express *his3* at the basal level but are unable to increase the level of expression in the appropriate physiological conditions. These define two regulatory sites located 32–41 and 80–100 bp upstream from the site of transcriptional initiation, and they strongly suggest that these regions are necessary for the positive regulation of *his3* expression. I consider the results in terms of models for the general control of amino acid biosynthesis.

his3 encodes imidazoleglycerol phosphate dehydratase (IGPD), an enzyme involved in histidine biosynthesis. Its expression is regulated coordinately with the genes involved in amino acid biosynthesis. When yeast cells are starved of histidine, methionine or tryptophan by adding appropriate inhibitors to the growth medium, the levels of IGPD increase about threefold (Table 1). In addition, *his3* expression is controlled in the expected manner by the *aas2* and *tra3* genes.

It is probable that *his3* is regulated solely as a function of general amino acid biosynthesis: first, IGPD levels do not depend on the presence or absence of histidine in the growth medium (Table 1). Second, growth conditions resulting in the selective induction of histidine biosynthetic enzymes have never been found. Third, mutations selected for defective regulation of histidine enzymes always affect other amino acid biosynthetic enzymes also[3]. These experiments, which fail to demonstrate histidine-specific regulation in yeast, are identical to those that clearly establish such control in *Salmonella typhimurium*.

The mapping positions of 18 mutations of the cloned *his3* gene are presented in Table 2. Each mutation deletes sequences (ranging between 9 and 66 bp) within the untranscribed region located 6–138 nucleotides upstream from the 5′ end of the gene. Using standard techniques, the mutant genes were introduced back into yeast cells so that the resulting strains contained one copy of the cloned DNA per cell at the normal *his3* chromosomal location (for experimental details see Fig. 1 legend). Strains harbouring these cloned mutant alleles were always grown in medium containing histidine so that *his3* expression was gratuitous for cell growth; inducing conditions were achieved by starving the cells of tryptophan. The IGPD levels are shown in Table 2. Two strains were examined for each *his3* allele. One strain contained all the original transforming DNA sequences; the other lacked the vector sequences and is equivalent to replacement of the original *his3*-532 mutation by the allele of interest (see Fig. 1 legend). The IGPD levels for both strains of any given mutant were always the same. Of the 18 mutations tested, 9 regulate *his3* expression properly. However, strains with any of the nine mutations produce IGP constitutively, that is, the enzyme levels are the same in normal conditions and during amino acid starvation. Thus, these nine mutations have the expected properties of *his3* regulatory mutations.

his3 regulatory sites are defined by comparing the DNA sequences of mutations that either regulate or fail to regulate *his3* expression. The simplest interpretation of the data assumes that: (1) a subset of the sequences deleted by a regulatory mutation is essential for normal regulation; and (2) sequences deleted by correctly regulated derivatives are unimportant. Such analysis, when applied to all the derivatives, leads to the simple and internally consistent description of *his3* regulatory elements illustrated in Fig. 2 and discussed below. It is difficult to disprove alternative explanations for the phenotype of any given mutation, and more complex models can be advanced to explain the data.

The extent of *his3*-Δ22 indicates a regulatory site that includes a sequence between 32 and 41 bp upstream from the start of transcription (nucleotides −32 to −41). Deletion of this region accounts for the regulatory defects of five other mutations (*his3*-Δ20, Δ21, Δ23, Δ24 and Δ25). As yet, the DNA sequences sufficient to constitute this regulatory site are not well mapped. However, sequences further upstream than −52

Table 1 Regulation of the *his3* gene

Strain	Genotype	Complete	−His	−Trp	+AT	+Eth
KY29	*trp1*-289 *his3*$^+$	1.0	1.0	3.0	3.0	3.2
KY137	*trp1*-289 *his3*-532	<0.1	NG	<0.1	NG	NT
KY107	*aas2*-5039 *his3*$^+$	1.0	1.1	NT	1.5	1.3
KY108	*tra3*-1 *his3*$^+$	3.0	3.0	NT	3.5	3.4

The yeast strains used were: KY29 (*mat-a ura3*-52 *trp1*-289) isolated by M. Thomas as strain M1-2B; KY137 (*mat-a ade2*-1 *ura3*-1 *ura3*-2 *trp1*-289 *his3*-532 *can1*$^-$) isolated by S. Scherer; KY107 (*mat-a aas2*-5039) and KY108 (*mat-a tra3*-1) both obtained from G. Fink[3]. Because *tra3*-1 renders strains temperature-sensitive for growth, all cultures were incubated at 27 °C with shaking. Complete medium contained 2% glucose, 0.67% yeast nitrogen base without amino acids (Difco), and 0.1 mg ml^{-1} each of histidine, tryptophan, uracil and adenine. In these conditions, the doubling time was 2 h for all strains except KY108, which doubled every 3 h. Strains incubated in complete medium lacking histidine (−His) grew at the same rate, except for KY137 which did not grow (NG). Cells grown in either of these non-starvation conditions were collected in the middle of exponential growth ($A_{600} = 2$ or $\sim 2\times 10^7$ cells ml^{-1}). To achieve amino acid starvation, cells were first grown in complete medium until A_{600} was ~1.0, concentrated by centrifugation, washed once with distilled water, and then resuspended in complete medium that either lacked tryptophan (−Trp), lacked histidine but contained 10 mM aminotriazole (+AT), or contained 20 μM ethionine (+Eth). The resuspended cells were incubated for a further 6–9 h; the cells undergo an average of one more cell division before their growth is arrested as a result of Trp, His or Met starvation. To determine the levels of IGPD activity, cells were collected, washed twice with water, permeabilized with 1.5% chloroform at 37 °C for 20 min, pelleted by centrifugation, and resuspended in 0.1 M triethanolamine, pH 7.7. The enzyme assay was performed as described previously[12] with minor modifications (to be published elsewhere). The enzyme levels are normalized to both the number of cells assayed and to the amount of absorbance at 290 nM released after chloroform treatment. The activity levels shown are relative to those of wild-type yeast grown in complete medium (defined as 1.0). For enzyme levels around 1.0, the error is ~10%.

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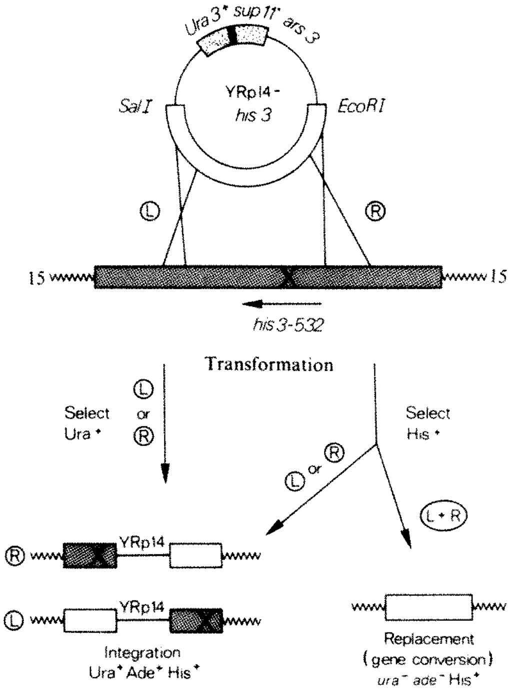

Fig. 1 Determining the phenotypes of cloned *his3* derivatives. The wild-type *his3* DNA used in these experiments (Sc2605) is a 6.1-kilobase pair (kb) fragment generated by *Eco*RI and *Sal*I cleavage[12]. In addition to the entire *his3* locus, it contains 2.9 kb flanking the 3′ end and 2.5 kb flanking the 5′ end. The isolation, haracterization and DNA sequence determination of the mutant derivatives of Sc2605 will be described elsewhere. The upper part of the figure shows the structure of *his3* hybrid molecules. All *his3*-containing DNA fragments (open boxes) were cloned into YRp14 (solid line with dotted boxes; see ref. 16), a vector containing the *ura3* gene, the ochre suppressing version of a tyrosine-inserting tRNA (*SUP11*), and *ars3*, a sequence that inefficiently causes hybrid molecules to replicate autonomously. YRp14 hybrid DNAs were introduced into KY137, a strain containing the ochre suppressible allele *ade2*-1, nonrevertible alleles *ura3*-1,2 and *his3*-532, and the revertible *trp1*-289 allele. Chromosome 15 of this strain is indicated by a wavy line below the YRp14-*his3* hybrid molecule. The shaded box indicates the 6.1-kb region homologous to the cloned *his3* derivatives; X marks the location of the *his3*-532 mutation[13]. Ura$^+$ or His$^+$ transformants were selected after 5 days of growth at 30 °C. All cloned *his3* fragments produced an equal number of His$^+$ and Ura$^+$ transformants for a given amount of DNA, except for Sc2884 (*his3*-Δ38) which produced no His$^+$ colonies. Essentially all such transformants were mitotically stable for the marker selected in the initial transformation event, indicating that the transforming sequences recombined with genomic DNA. Transformants containing autonomously replicating hybrid molecules were obtained only after ≥10 days. This is probably due to the inefficiency of *ars3*[16] and to lethality associated with multiple copies per cell of tyrosine-inserting suppressor tRNAs[14]. The figure shows recombination with the *his3* locus of chromosome 15. The L cross-over occurs to the left of the *his3*-532, while the R cross-over occurs to the right of this allele. The resulting structures are shown at the bottom. The YRp14 vector sequences which include *ura3*$^+$ *sup11*^{o} *ars3* are indicated by a solid line. Almost all the Ura$^+$ transformants were also His$^+$ Ade$^+$ while the His$^+$ transformants fell into two classes. The class of His$^+$ transformant that was also Ura$^+$ Ade$^+$ is genotypically equivalent to the Ura$^+$ transformants. In this case, the entire YRp14 hybrid molecule has integrated into the genome. As most of the homology between the transforming DNA and the genome is *his3* DNA, integration probably occurs at the *his3* locus; this was true for all cases tested explicitly. The transformants that arise by integration are due to a single L or R cross-over. The other class of His$^+$ transformant is *ade*$^-$ *ura*$^-$ and results from replacement of the *his3*-532 allele by the transforming allele, probably via gene conversion[15]. This class is equivalent to the double (L+R) cross-over. Both classes of transformants should have one copy of the transforming DNA per cell. For the integration class, multiple copies of the *SUP11*-containing transforming DNA per haploid genome should be lethal and for the replacement class, it is difficult to envisage mechanisms that could result in multiple copies of *his3* and none of *ura3*. Indeed, hybridization analysis supports the above contentions. It is possible that cross-overs occur between the *his3*-532 and the cloned *his3* alleles—this would generate either the wild-type allele and/or the double mutant. However, as these alleles are very close (less than 250 bp; see ref. 13) recombination between them is rare[5]. In this regard, note that the phenotype of each allele was tested in two independent transformants.

or downstream from −32 are unlikely to be involved because both *his3*-Δ26 (which deletes nucleotides −53 to −80) and *his3*-Δ18 (nucleotides −32 to −21 deleted) are regulated properly.

The structures of the remaining deletion mutations suggest a second regulatory site: *his3*-Δ28 (nucleotides −106 to −53 deleted) and *his3*-Δ29 (nucleotides −106 to −73 deleted) fail to regulate *his3* expression. However, the related pair that differ only in the upstream breakpoint (*his3*-Δ26 lacking −80 to −53 and *his3*-Δ27 lacking −80 to −73) are regulated properly. By subtraction, these results strongly suggest a second regulatory site that includes at least a subset of the sequences between −80 and −106. The upstream boundary of this site is defined further by deletions 32–35. These mutations remove sequences between −101 and −137, but do not prevent *his3* regulation. Some of them do seem, however, to have reduced basal levels of expression.

Thus, there seem to be at least two regulatory elements for *his3* expression separated by at least 30 bp. The downstream element maps between −32 and −52 and includes a region localized between −32 and −41, the upstream element maps somewhere between −80 and −100.

It is worth comparing the sequence of the *his3* regulatory regions defined here[4,7] with the nucleotide sequence of *his4*[8], a gene that is co-regulated with *his3*. The *his3* sequence between nucleotides −44 and −31, TATATAAAGTAATG, strongly resembles the *his4* sequence TATATAATAGATATG, which is located 46–60 bp before the mRNA start point[8]. These sequences contain 12 out of 14 identical base pairs if a single base pair is allowed to 'loop out'. With regard to the upstream regulatory element, the *his3* sequence between −97 and −90, ATGACTCT, shares 7 out of 8 base pairs with the *his4* sequence ATGACTAT located between −86 and −79. Furthermore, highly conserved variants of this sequence are found at *his3* sequences −139 to −132 (ATGCCTCG) and −178 to −171 (ATTACTCT) and *his4* sequences −137 to −130 (GTGACTCA) and −181 to −174 (GTGACTCC). This striking sequence homology supports the view that these regions are important in *his3* regulation.

Either of two simple models could account for the increased *his3* expression in conditions of amino acid starvation. In one, the gene interacts with positive regulatory factors that stimulate expression above the basal level; in the other model, the *his3* gene interacts with negative regulatory factors that maintain the basal level, and *his3* induction occurs by the inactivation of these factors. Proof of a positive and/or negative mode of *his3* regulation depends on the properties of purified regulatory factors with respect to the appropriate target DNAs. Nevertheless, evidence for the nature of *his3* regulation can be inferred from the phenotypes of regulatory mutations at the *his3* locus.

By definition, mutations at a positive regulatory site would result in basal levels of *his3* expression in all growth conditions. Indeed, five mutations (deletions 20, 22, 23, 24 and 28) have such a phenotype—this strongly suggests that at least some of the sequences deleted by these mutations comprise a positive regulatory site. The other four regulatory mutations result in constitutive expression below the basal level; thus it is difficult to assess their phenotype in terms of positive or negative control.

As for the possibility of negative control, so far I have obtained no *his3* mutations that result in constitutive *his3* expression at the induced level; these would be expected if the gene contains a site of negative regulation analogous to the

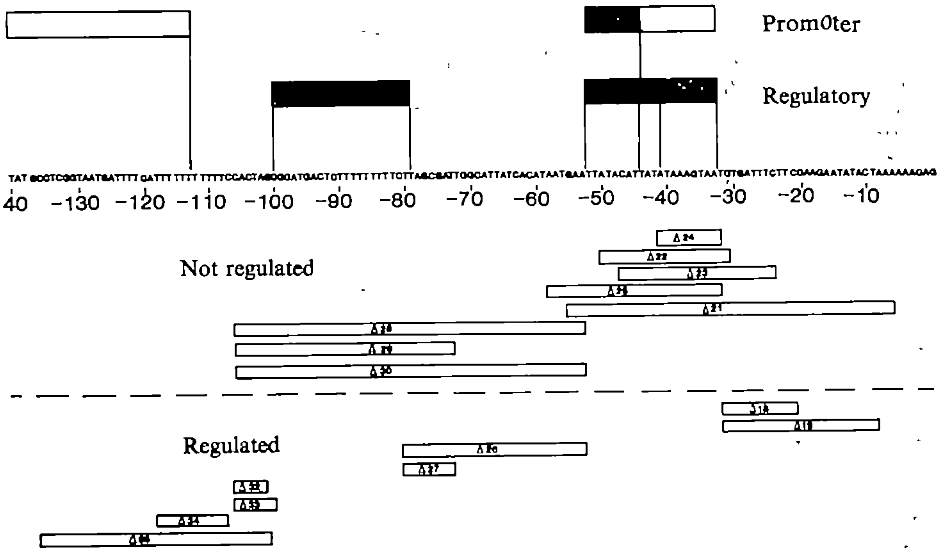

Fig. 2 *his3* promoter and regulatory elements. The open boxes on the top line indicate the extent of promoter elements defined previously[5,6] and the shaded boxes on the second line indicate the location of positive regulatory elements described here (the black regions within these boxes indicate sequences that are essential but not necessarily sufficient for promoter or regulatory function) These elements are placed with respect to the coding strand of the 5′ flanking region (nucleotides +1 to −140 defined with respect to the start of transcriptional initiation). The mRNA start was previously defined to an accuracy of ±1 bp[7]. The location of the deletion mutations used in this study are indicated below the DNA sequence. Deletions above the broken line do not allow for properly regulated *his3* expression, while the deletions below the line are regulated correctly.

operator of the *Escherichia coli* lactose operon. Nevertheless, it is possible that the five mutations expressing *his3* constitutively at the basal level could delete a negative regulatory site and simultaneously reduce promoter activity threefold. However, the principal advantage of deletion analysis is the ability to eliminate DNA sequences and hence genetic functions. For example, nine mutations described here eliminate regulatory behaviour, and 30 mutations (described in refs 5, 6) eliminate detectable *his3* expression. Thus, it seems unlikely that the five critical deletions fortuitously result in the same quantitatively minor defect, particularly considering the fact that nine deletions having full or partially reduced *his3* levels in normal conditions regulate the gene properly. Therefore, the mutants strongly support a positive control mechanism for *his3* expression as opposed to a negative one.

Table 2 Structures and phenotypes of *his3* derivatives

Fragment	*his3* allele	Upstream end point	Junction sequence	Downstream end point	Basal level	Induced level
Sc2605	Wild type	—	—	—	1.0	3.0
Sc2854	Δ18	−32	RI	−21	0.5	1.5
Sc2857	Δ19	−32	RI	−32	0.7	2.0
Sc3101	Δ20	−43		−31	1.1	1.0
Sc3102	Δ21	−56		−6	0.2	0.2
Sc3110	Δ22	−50		−31	1.2	1.3
Sc3111	Δ23	−47		−24	0.8	1.0
Sc3112	Δ24	−41	CC	−32	1.1	1.4
Sc3113	Δ25	−58	TCC	−32	0.2	0.2
Sc3121	Δ26	−80	RI	−53	0.9	2.7
Sc3122	Δ27	−80	RI	−73	0.6	1.5
Sc3125	Δ28	−106	RI	−53	1.1	1.0
Sc3126	Δ29	−106	RI	−73	0.2	0.3
Sc3129	Δ30	−119	RI	−53	0.2	0.1
Sc3138	Δ31	−106	RI	−109	1.0	3.1
Sc3159	Δ32	−106		−102	0.8	2.9
Sc3160	Δ33	−106		−100	1.2	3.2
Sc3161	Δ34	−118		−107	0.2	0.6
Sc3165	Δ35	−136		−101	0.5	1.2
Sc2884	Δ38	−80	RI	−32	<0.1	<0.1

All Sc fragments (first column) are derivatives of Sc2605 (see Fig. 1 legend). The *his3* deletion allele numbers and the end points with respect to the start of transcription (determined elsewhere by direct DNA sequencing) are indicated (columns 2 and 3). In some cases, there are junction nucleotides at the site of deletion (RI, *Eco*RI octanucleotide linker, 5′-GGAATTCC-3′). For each *his3* allele tested, 1 Ura$^+$ transformant (containing one integrated copy of the transforming DNA) and 1 His$^+$ transformant (in which the *his3*-532 allele is replaced with the transforming *his3* allele) were grown (see Fig. 1 legend for details). These always gave similar results; the values shown represent the averages of the two strains. To obtain the basal IGPD levels for a given mutation, strains were grown in complete medium. To obtain enzyme levels in starvation conditions, tryptophan was removed from the medium (see Table 1 legend for the methodology and reliability of the assay).

The data presented here suggest that the *his3* regulatory region consists of two separable positive control sites, each of which can be destroyed without apparently impairing promoter function. The conclusions represent the simplest formal description of the data, but have no bearing on the molecular mechanism by which *his3* expression is modulated in response to amino acid starvation. As induction of IGPD activity is accompanied by an increase in *his3* mRNA[9], it is likely that regulatory sites are involved in transcriptional initiation; this, however, has not been proved.

The key question is the nature of the 'positive regulatory factor'. Some possibilities are: (1) a protein product(s) that binds specifically to the sequence(s) defined as regulatory elements; (2) a conformational or covalent alteration in yeast RNA polymerase II that senses the regulatory sequences; (3) a structural feature of chromatin; and (4) a structural change in the *his3* transcript that affects its stability or translatability. Biochemical analysis of strains harbouring 'regulation-defective' *his3* alleles should be useful for distinguishing among these and other molecular models. Preliminary results with wild-type cells suggest that the chromatin structure and the mRNA structure are unchanged during amino acid starvation (ref. 4 and K.S., in preparation).

In considering molecular models for *his3* regulation, it is worth comparing the results obtained here with those of an analogous situation in *E. coli*. Amino acid starvation of *E. coli* results in increased expression of amino acid biosynthetic genes and decreased expression of those encoding stable RNA species such as the tyrosine-inserting tRNA. A mutation of this tRNA gene that changes sequences between the downstream promoter element (the Pribnow box) and the start of transcription retains promoter function but is regulated incorrectly[10]. Further, *in vitro* transcription of this gene using purified components suggests that regulation of the tyrosine tRNA promoter is mediated by the interaction of *E. coli* RNA polymerase with guanosine tetraphosphate[11]. It is striking that the *his3* regulatory site between −32 and −41 also maps between (or overlaps with) the TATA box region and the mRNA start. Whether this means that regulation in yeast occurs by a mechanism similar to that used by *E. coli* remains to be determined.

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Isolation of a mouse pseudo tRNA gene encoding CCA—a possible example of reverse flow of genetic information

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Transfer RNA genes offer an interesting system for studies of gene regulation. From experiments conducted with tRNA genes from eukaryotes, it seems that transcriptional regulatory regions are contained within the mature coding sequence of the DNA[1–4]. In tRNA genes of some yeast, *Xenopus* and *Drosophila*, intervening sequences located within the anticodon loop have been identified[5–8], thus adding a new dimension to tRNA expression. In contrast to the lower eukaryotes, relatively few mammalian tRNA genes have been studied. The genes obtained so far have been identified via cloned heterologous fragments, or labelled tRNA probes[9–11]. We sought to use synthetic oligodeoxyribonucleotide probes to isolate mouse phenylalanyl tRNA genes. We report here the isolation of a segment of DNA that is completely homologous to our 19-mer probe, as well as 38 nucleotides corresponding to the 3′ end of tRNA$^{\mathrm{Phe}}$. The 5′ end of the gene is not present in our 1.7 kilobase clone. In addition, this pseudo gene has an encoded CCA. Since CCA is added post-transcriptionally to all known eukaryotic tRNAs for which genes have been identified, we believe this pseudogene to be representative of RNA encoded information going back into DNA.

We chose to isolate a mouse gene coding for tRNA$^{\mathrm{Phe}}$ by using as a probe a synthetic oligodeoxyribonucleotide 19-mer complementary to a portion of the known mammalian tRNA$^{\mathrm{Phe}}$ sequence[12,13] (see Fig. 1*a*). The strategy utilized was the screening of a partially digested mouse DNA library inserted at the *Hin*dIII site of pBR322. This plasmid library was used to transform *Escherichia coli* and selection was made for ampicillin resistant colonies. The colonies were screened for the tRNA$^{\mathrm{Phe}}$ gene containing inserts as described in Fig. 1 legend. Figure 1*b*, *c* shows autoradiographs of colony hybridizations with the synthetic probe. Four putative positives were obtained by the colony screening, two of which gave positive hybridization results on rescreening. These two clones appeared to be siblings as judged by DNA restriction (not shown). One of these plasmids, designated as pMTF3, was chosen for further studies.

Figure 2*a* presents ethidium bromide stains of restriction endonuclease digestions of pMTF3. Digestion with *Hin*dIII yielded a 3.4 kilobase pair (kbp) vector band, and 1.2 and 1.7 kbp insert bands. It is not known if the two *Hin*dIII inserts were due to ligation of partially digested DNA or were the result of ligation of two separate fragments. To test which of the two *Hin*dIII fragments contained the sequence homologous to the 19-mer probe, the DNA in the gel was transferred to nitrocellulose[14] and then hybridized with the ^{32}P-labelled synthetic 19-mer probe. These results demonstrated that only the 1.7 kbp *Hin*dIII fragment contained a sequence homologous to the probe (Fig. 2*a*). Digestion of pMTF3 with *Ava*I (CPyCGPuG) gave three stained fragments that had summated fragment lengths equal to undigested pMTF3. No hybridization with the 19-mer probe was seen in the *Ava*I digest (Fig. 2*a*). This same lack of hybridization was observed with *Hpa*II (CCGG) and *Sma*I (CCCGGG) digests (data not presented). This failure to detect hybridization with the 19-mer probe in the *Ava*I, *Hpa*II and *Sma*I digests is due to the presence of these sites in the middle of the region of complementarity to the 19-mer probe (Fig. 1).

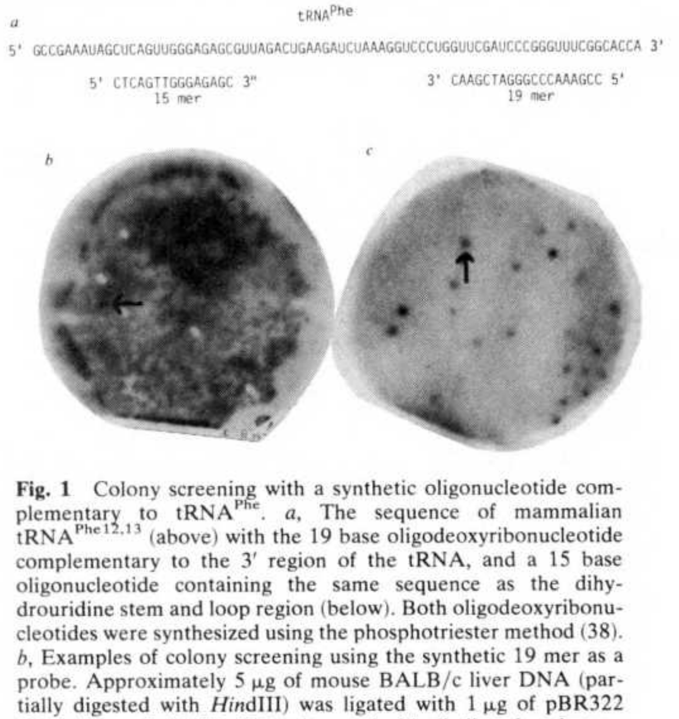
a
tRNA$^{\mathrm{Phe}}$
5' GCCGAAAUAGCUCAGUUGGGAGAGCGUUAGACUGAAGAUCUAAAGGUCCCUGGUUCGAUCCCGGGUUUCGGCACCA 3'
5' CTCAGTTGGGAGAGC 3"
15 mer
3' CAAGCTAGGGCCCAAAGCC 5'
19 mer
b
c

Fig. 1 Colony screening with a synthetic oligonucleotide complementary to tRNA$^{\mathrm{Phe}}$. *a*, The sequence of mammalian tRNA$^{\mathrm{Phe}}$[12,13] (above) with the 19 base oligodeoxyribonucleotide complementary to the 3′ region of the tRNA, and a 15 base oligonucleotide containing the same sequence as the dihydrouridine stem and loop region (below). Both oligodeoxyribonucleotides were synthesized using the phosphotriester method (38). *b*, Examples of colony screening using the synthetic 19 mer as a probe. Approximately 5 μg of mouse BALB/c liver DNA (partially digested with *Hin*dIII) was ligated with 1 μg of pBR322 DNA digested with *Hin*dIII and treated with alkaline phosphatase. This DNA mix was used to transform *E. coli* strain MC1061[39] from which approximately 1 × 10^5 transformants were obtained, about 90% of which were tetracycline sensitive. This represents approximately 15–20% coverage of the genome. These colonies were first replicated to dry, sterile nitrocellulose filters. The colonies on nitrocellulose were used to make replicas onto Whatman 541 paper[40]. The nitrocellulose replicas were incubated on L-agar plates containing ampicillin (30 μg ml^{-1}) and used as masters for retrieving positively hybridizing colonies. The Whatman 541 replicas were treated according to Gergen *et. al.*[40], and hybridized with 75 pmol (500 × 10^6 c.p.m.) ^{32}P-polynucleotide kinase labelled 19-mer. The hybridizations were carried out overnight at 45 °C in 3–5 ml per filter of 6 × SET (30 mM Tris-HCl pH 7.5, 0.9 M NaCl, 6 mM EDTA), 5 × Denhardt's medium, 0.5% Nonidet P40 following 2–3 hours of prehybridization in the same buffer. The filters were washed in 6 × SET at progressively higher temperatures, beginning with 20 °C, until background hybridization was satisfactorily eliminated[41]. The final wash was at 45 °C. After washing, the filters were autoradiographed at −70°C on Kodak XRP-1 film with a DuPont Lightning Plus intensifying screen. *b*, A small area (approximately 1 cm) corresponding to the region of a positively hybridizing colony was scraped, diluted, replated and rescreened with the 19-mer probe. *c*, Positively hybridizing, isolated single colonies from the rescreening were used for DNA isolations.

Using standard double and triple restriction endonuclease digestions, a restriction site map for pMTF3 was constructed (Fig. 2*b*). Since the *Sma*I site (Figs 1*a*, 2*b*) is in the centre of the region of complementarity with the 19-mer probe, the DNA sequences including and surrounding this site were determined by the chemical cleavage method[15].

The DNA sequencing results are summarized in Fig. 3. From these data we find a region of perfect homology to the probe. Comparison to the known tRNA$^{\mathrm{Phe}}$ sequence (Fig. 3) demonstrates that 38 contiguous nucleotides of the tRNA$^{\mathrm{Phe}}$ 3′ region are included in the cloned segment. This homology diverges within the region corresponding to the tRNA$^{\mathrm{Phe}}$ anticodon loop.

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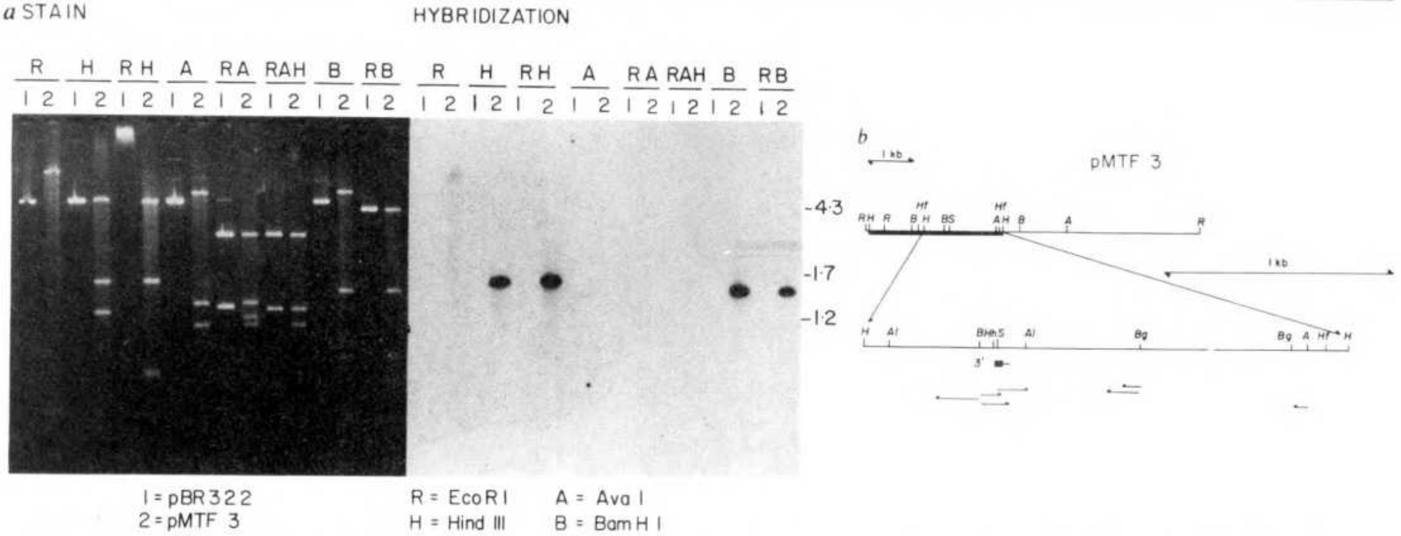

Fig. 2 *a*, Restriction endonuclease digestions and hybridization of the 19-mer probe to pMTF3. Plasmid DNA from pMTF3 was prepared and purified by ethidium bromide CsCl gradients[42]. This DNA was then treated with various restriction enzymes (as shown) under conditions described by Bethesda Research Laboratories (BRL). Samples were then subjected to electrophoresis through an 0.8% agarose gel. The gel was stained and photographed. Markers of *Hin*dIII, *Eco*RI and *Hpa*II digested λ DNA were run in adjacent channels (not shown). Transfer of the DNA to nitrocellulose was by the method of Southern[14]. Prehybridization of the filter was at 42 °C in 6×SSC plus 5×Denhardt's medium for 12 hours[34]. Hybridization was for 36 hours with 6×SET, 5×Denhardt's, and 50×10^6 c.p.m. ^{32}P-labelled 19 mer. After hybridization, the filter was washed four times for 1 hour each at 42 °C in 6×SSC and exposed to Kodak XAR-5 film at −70 °C with DuPont Lightning Plus intensifying screens. The faint hybridization of the 19-mer probe to the *Eco*RI digest was the result of poor transfer from the gel to nitrocellulose. *b*, A restriction site map of pMTF3. Shown is the pBR322 vector linearized at its *Eco*RI site and emphasizing the *Hin*dIII inserts. The position and polarity of the tRNA$^{\mathrm{Phe}}$ psuedogene sequence is shown (■). Also depicted are the restriction sites used for labelling and the direction of DNA sequencing. Restriction sites shown are R, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; S, *Sma*I; A, *Ava*I; Al, *Alu*I; Bg, *Bgl*II; Hf, *Hin*fI; and Hh, *Hha*I.

No sequence completely homologous to the 5′ end of tRNA$^{\mathrm{Phe}}$ was found within 100 base pairs from this point of divergence (Fig. 3).

In an attempt to localize the 5′ end of tRNA$^{\mathrm{Phe}}$, a synthetic oligodeoxyribonucleotide containing the dihydrouridine stem and loop region sequences of tRNA$^{\mathrm{Phe}}$ (Fig. 1) was chemically synthesized. This 15-mer was used to probe DNA from pMTF3 as described above. The only hybridization observed generated a weak signal with a restriction fragment which maps to the 3′ side (downstream) of the region of the 19-mer homology (data not presented). DNA sequence analyses of this region did not demonstrate any region of complete homology with the 15-mer probe nor with the known tRNA$^{\mathrm{Phe}}$ sequence.

The presence of the 3′ end of tRNA$^{\mathrm{Phe}}$ and the apparent absence of the 5′ end suggests a pseudogene. The presence of an encoded 3′ terminal CCA in our cloned segment is also quite unusual. An examination of published sequences for over 50 nuclear tRNA genes from such diverse higher eukaryote sources, such as, *Bombyx*, *Drosophila*, *Xenopus*, rat and man, reveals no encoded CCAs[7,10,11,16–23]. Furthermore, none of the 22 mouse[45] or human[24] mitochondrial tRNA genes encode CCAs. All available evidence suggests CCAs are added post-transcriptionally in eukaryotes. Several other interesting features of the cloned mouse DNA segment in pMTF3 must be considered. The position at which the cloned sequence diverges from the known tRNA$^{\mathrm{Phe}}$ sequence is one base pair off from the site at which an intervening sequence has been localized in some eukaryotic tRNA genes[5–8]. The 5′ end of tRNA$^{\mathrm{Phe}}$ has some points of weak homology to pMTF3 in the region sequenced. These sequences are noted in Fig. 3. We do not know if these similarities represent divergence in the 5′ region or if they are random. The nucleotide sequence presented in Fig. 3 has been analyzed for tRNA-like secondary structures using the computer program developed by Staden[25]. No structures capable of forming stable tRNA secondary structures with the appropriately conserved nucleotides were predicted by this search.

In screening 15–20% of the mouse genome (see Fig. 1 legend), we obtained a single tRNA$^{\mathrm{Phe}}$ related recombinant (pMTF3). This yields a minimum estimate of five tRNA$^{\mathrm{Phe}}$ related sequences per haploid genome. We have not yet isolated the complete tRNA$^{\mathrm{Phe}}$ gene. This could be due to a lower than predicted gene copy number, or a bias in our cloning approach.

To determine if the cloned tRNA$^{\mathrm{Phe}}$ pseudogene was indeed derived from mouse, the *Hin*dIII 1.7 kbp insert fragment was used as a probe to mouse genomic DNA. The results presented in Fig. 4 show a smear of hybridization spread over the entire

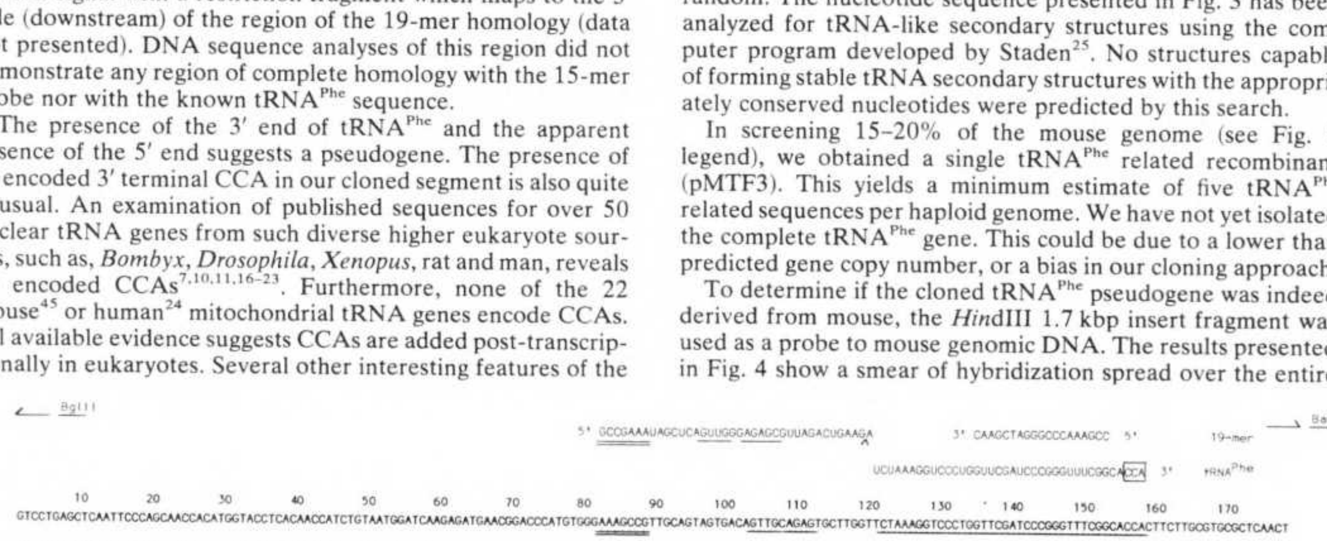
BglII
5' GCCGAAAUAGCUCAGUUGGGAGAGCGUUAGACUGAAGA
3' CAAGCTAGGGCCCAAAGCC 5'
19-mer
BamHI
UCUAAAGGUCCCUGGUUCGAUCCCGGGUUUCGGCACCA 3'
tRNA^Phe
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170
GTCCTGAGCTCAATTCCCAGCAACCACATGGTACCTCACAACCATCTGTAATGGATCAAGAGATGAACGGACCCATGTGGGAAAGCCGTTGCAGTAGTGACAGTTGCAGAGTGCTTGGTTCTAAAGGTCCCTGGTTCGATCCCGGGTTTCGGCACCACTTCTTGCGTGCGCTCAACT
CAGGACTCGAGTTAAGGGTCGTTGGTGTACCATGGAGTGTTGGTAGACATTACCTAGTTCTCTACTTGCCTGGGTACACCCTTTCGGCAACGTCATCACTGTCAACGTCTCACGAACCAAGATTTCCAGGGACCAAGCTAGGGCCCAAAGCCGTGGTGAAGAACGCACGCGAGTTGA
a
e e
a
b b
c c c c c
d
d
f f
g h g h g g

Fig. 3 DNA sequence of pMTF3 in the region of 19-mer homology. The pMTF3 sequence is aligned with the known mammalian tRNA$^{\mathrm{Phe}}$ sequence using the tRNA$^{\mathrm{Phe}}$ CCA as the start point. Nucleotides in the pMTF3 sequence which are present in the corresponding position of tRNA$^{\mathrm{Phe}}$ are underlined. Note that the 5′ terminal tRNA$^{\mathrm{Phe}}$ sequence GCCGAAA is present as an inverted segment at the same relative position in pMTF3. Other areas of interest, such as direct (→ →), inverted (→ ←), pallindromic repeats (← →), and the position at which an intervening sequence would be expected to occur (∧) are indicated.

genomic restriction profile for each of the enzymes or combinations tested. Hybridizations done under identical stringent conditions with mouse ribosomal gene probes yielded specific banding patterns[26]. The broad hybridization distribution of the 1.7 kbp. *Hin*dIII band to numerous genomic fragments indicates the presence of repetitive elements in the clone. In a separate experiment (not shown), mouse genomic DNA was hybridized to restriction digests of pMTF3. The data indicate that there are repeated sequences located on both sides of the pseudo gene. The vector DNA showed no homology to mouse. It is not known whether these elements are tRNA genes or members of a mouse family of repeated sequences, or both.

The origin and functional significance, if any, of all psuedogenes is of interest. In *E. coli* several psuedogene sequences for tRNA$^{\text{Tyr}}$ which contain partial, but non-degenerate homology with the mature coding sequences are found distal to the mature coding sequences[27]. Some biological function is suggested since they are stably maintained in the chromosomes of several *E. coli* K12 strains[28] and are included in the tRNA$^{\text{Tyr}}$ containing primary transcripts[29]. Little is known concerning any possible functional role of eukaryotic pseudogene sequences. The *Xenopus* 5S RNA pseudogenes are 85% homologous with the 5S genes[30]. In one report the pseudogene sequence was observed to be transcribed following injection of the cloned DNA segment into *Xenopus* oocytes[31]. It is not clear whether these sequences are transcribed *in vivo*. Transfer RNA pseudogenes which share degenerate or incomplete homologies with the mature coding sequences, have been described for *Drosophila* tRNA$^{\text{met}}$ (ref. 20) and rat tRNA$^{\text{Gly}}$ and tRNA$^{\text{Glu}}$ (ref. 23). Unlike the tRNA$^{\text{Phe}}$ pseudogene sequence reported here, neither the *Drosophila*, nor rat pseudogene sequences contain an encoded CCA. Mammalian pseudogenes for globin, tubulin and immunoglobin have been reported which have structural and sequence features suggesting an RNA origin[32–34].

Any model used to explain the origin of the tRNA$^{\text{Phe}}$ pseudogene sequence must take into account the facts that this sequence is completely homologous with 38 3′-terminal nucleotides of tRNA$^{\text{Phe}}$ and has an encoded CCA. This region of homology is more extensive than the tRNA priming sequences found in retroviral DNAs (~18 nucleotides)[35], although we cannot eliminate a retroviral origin for this pseudogene sequence. Since we do not know the structure of the tRNA$^{\text{Phe}}$ gene itself, we can only speculate based on analogy with other eukaryotic tRNAs that the CCA is added post-transcriptionally, and therefore is not encoded.

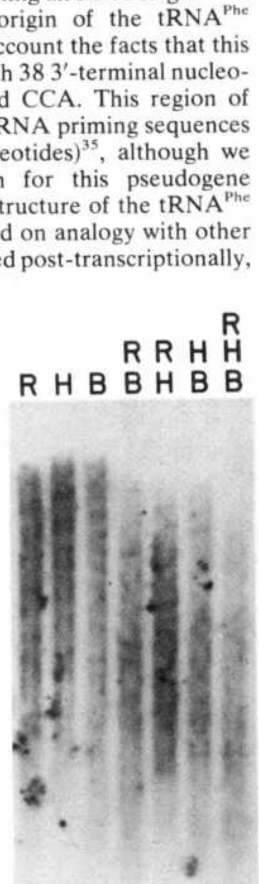

Fig. 4 Hybridization of pMTF3: *Hin*dIII A band to mouse genomic restriction digests. DNA from a male BALB/*c* kidney was prepared and digested with various combinations of *Eco*RI, E; *Hin*dIII, H; or *Bam*HI, B. Completion of the digests was verified using λ DNA as an indicator. The samples were then subjected to electrophoresis through 0.8% agarose, stained and photographed. Markers of lambda *Hin*dIII and λ *Eco*RI were run in adjacent channels. The DNA in the gel was then transferred to nitrocellulose by the method of Southern[14]. The probe was prepared by first digesting pMTF3 with *Hin*dIII, separating out the fragments depicted in Fig. 2, and recovering the *Hin*dIII A band via electroelution. The recovered band (0.5 μg) was then nick translated[43] with [α-^{32}P]dCTP. Hybridization of the probe to the filter was by the method of Wahl *et al.*[44], with modifications of Reilly *et al.*[26]. The final wash was at 65 °C in 0.2 × SSC. Autoradiography was as described for Fig. 2.

The presence of an encoded CCA suggests that the information of the mature tRNA was reverse transcribed and integrated into the mouse genome. A similar phenomenon is suggested for tubulin[33] and immunoglobulin pseudogenes[34]. We do not know the mechanisms by which RNA characteristics, such as poly(dA) stretches, attached to intron-less coding regions (as seen in tubulin and globin pseudogenes) or the encoded CCA in pMTF3 arose. To explain the origin of such pseudogene sequences, mechanisms of the reverse transcription and genomic integration of the segments must be understood. In the absence of any clearly identified primer, we find it difficult to understand how a reverse transcript of only the 3′ end of tRNA$^{\text{Phe}}$ arose. With regard to integration of this pseudo-gene into the genome, repeated family sequences could be involved. The recent discovery of mammalian nuclear extrachromosomal circular DNA containing *Alu* family sequences suggests a possible intermediate[37]. In the case of several small nuclear RNA pseudogenes, the pseudogene sequences are flanked by short (16–19 bp) direct repeats[36]. We do not observe these types of repeats flanking the tRNA$^{\text{Phe}}$ pseudogene, although pMTF3 does contain repetitive sequences (Fig. 4). Any further insight into the origin of this pseudogene must await isolation and analysis of the complete tRNA$^{\text{Phe}}$ gene.

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Effect of interferon on protein glycosylation and comparison with tunicamycin

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The present wide interest in the mechanism of action of interferon (IFN) stems from its antiviral and anticancer potential. Possibly one of the more interesting insights into the antiviral action of IFN was recently described by Maheshwari *et al.*[1]. These authors, including one of us (K.O.), reported that IFN-treated cells release viral particles with low infectivity, and that this low infectivity seems to be related to the reduced amount of membrane protein glycosylation. The implications of this suggestion are exciting because inhibitors of glycosylation have antiviral properties, and also selectively kill transformed cells (see ref. 2 for review). An effect of IFN on the sysnthesis and cell-surface expression of glycoproteins could have an important role in its antiviral, anticancer and immunoregulatory actions. We have examined, for the first time, the effect of IFN on glycosylation of cellular proteins. We used murine fibroblasts (L-cells and NIH-3T3), plasmacytoma cells (P3 × 63 Ag8) and vesicular stomatitis virus (VSV) to compare the action of IFN with tunicamycin (TM), a known inhibitor of glycosylation[2]. Our results indicate that IFN does not inhibit the glycosylation of cellular or viral glycoproteins. Cells treated with IFN do not produce nonglycosylated species of glycoproteins nor the 'glucose/glycosylation regulated proteins'[3] (GRPs), in contrast to cells treated with tunicamycin[4].

Fibroblasts were grown in Eagle's minimal essential medium (MEM) as described by Maheshwari *et al.*[1,5–8], and plasmacytoma cells in Dulbecco–Vogt[9] modified Eagle's medium containing high glucose (4,500 mg l^{-1}). Cells were infected with VSV at a multiplicity of 0.5–10 plaque forming units (PFU) per cell as previously described[1,5–8], and radiolabelled substrates were added 3 h post-infection. Plaque formation was determined according to standard assay procedure[10] with confluent cultures of L-cells.

First, we compared the effects of IFN and TM on the glycosylation of cellular protein by monitoring the rate and total incorporation of D-[U^{14}C]glucosamine and D-[2^{3}H]mannose into the total trichloroacetic acid-insoluble fraction of the cell. The results of a representative 48-h incorporation experiment are shown in Table 1*a*. It is apparent that none of the concentrations of IFN were effective in inhibiting glycosylation of cellular protein, in marked contrast to TM. Similarly, no differences were found in the rate of incorporation into cultures monitored at hourly intervals for up to 3 h. Likewise, IFN had no effect on the glycosylation of specific proteins as evident by inspection of the fluorogram shown in Fig. 1*a*. This conclusion is also supported by the finding that IFN-treatment does not induce the synthesis of the GRPs, in contrast to cells treated with TM (Fig. 1*b*, and refs 4, 11).

To investigate further the effect of IFN on the glycosylation of specific cellular glycoproteins, metabolically labelled fibronectin (FN) and immunoglobin G (IgG) were isolated by immunoprecipitation and analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Inspection of the fluorograms in Fig. 2 shows that IFN does not decrease the glycosylation of either protein as shown by similar incorporation of radiolabelled carbohydrate precursor and by lack of shift in electrophoretic mobility as would be expected if the proteins were depleted of carbohydrate[12].

To determine if treatment of cells with IFN had any effect on the size or structure of the oligosaccharide chains of an asparagine-linked cellular glycoprotein, glycopeptides of FN were isolated from control and IFN-treated 3T3 cells and subjected to exhaustive digestion with pronase. The glycopeptides were analysed by Bio-Gel P4 filtration, treatment with *endo-β-N-*acetylglucosaminidase H (*endo* H), and by their

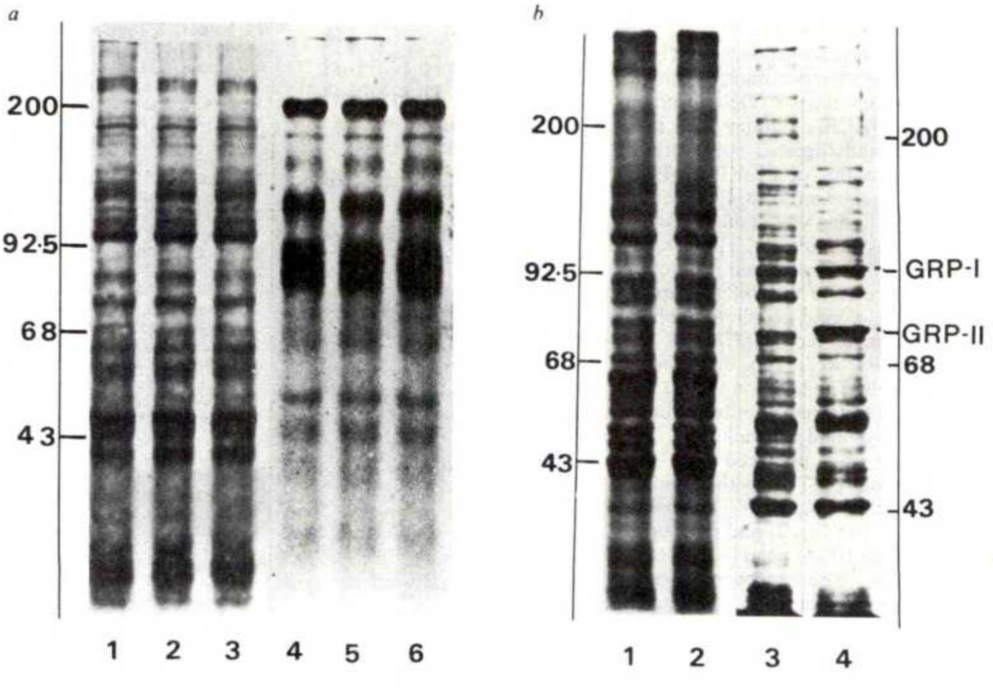

Fig. 1 *a*, Effect of IFN on glycosylation of plasmacytoma cellular proteins. Lanes 1, 2 and 3 represent ^{14}C-leucine (2 μCi ml^{-1}, specific activity 2 Ci mmol^{-1}) labelled cells incubated with 0, 30 and 250 U ml^{-1} of mouse fibroblast IFN (specific activity >4 × 10^{7} mouse reference units per mg protein) respectively. Lanes 4, 5 and 6 represent parallel cultures treated with similar amounts of IFN, but labelled with ^{14}C-D-glucosamine (5 μCi ml^{-1}, specific activity 238 mCi mmol^{-1}). Cells were preincubated with IFN for 16 h, then radioactive precursors were added and incubation was continued for 48 h. SDS-PAGE and fluorography were performed as previously described[23,24]. Protein was determined by the Lowry procedure[25] with bovine serum albumin standards. Similar results were obtained with IFN concentrations up to 1,000 U ml^{-1}, at 24 and 36 h, or with IFN purchased from Lee Biomolecular Research Laboratories. *b*, Effect of IFN and TM on the induction of the 'glucose/glycosylation regulated proteins'. Mouse L-cells were labelled with ^{14}C-leucine and incubated with IFN as described in Fig. 1 legend. Cells were pretreated for 6 h with TM before addition of ^{14}C-leucine. Lanes 1 and 2 represent cells treated with 0 and 30 U ml^{-1} IFN, respectively, and lanes 3 and 4 represent cells treated with 0 and 1.0 μg ml^{-1} TM, respectively. Similar results were obtained with IFN concentrations up to 1,000 U ml^{-1}.

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interaction with concanavalin A (Con A)–Sepharose. We found that glycopeptides from both the control and IFN-treated cells were resistant to *endo* H, reacted with Con A, and co-eluted on Bio-Gel columns with a molecular weight (~2,300) characteristic of the complex-type oligosaccharide (results not shown).

The above experiments provide strong evidence that IFN does not significantly alter the carbohydrate moieties of asparagine-linked cellular glycoproteins.

To determine if VSV produced in the presence of IFN contain surface carbohydrates, viral particles isolated from control and IFN-treated cultures were examined with respect to their specific binding to Con A–Sepharose. If viral particles released from IFN treated cells are depleted of the carbohydrate moiety on glycoprotein G, then they should not bind to Con A.

Table 1 Effect of tunicamycin and interferon on glycosylation of cellular protein (*a*) infectivity of VSV (*b*) and binding of VSV to concanavalin A–Sepharose (*c*)

a, Glycosylation			
Tunicamycin Precursor	[TM] (μg ml^{-1})	c.p.m. per μg protein	% Inhibition
^{14}C-leucine	0	1,123	0
(2 μCi ml^{-1})	0.05	1,309	0
	0.10	1,174	0
	0.50	1,060	12
	1.00	929	23
^{3}H-mannose	0	422	0
(5 μCi ml^{-1})	0.05	437	0
	0.10	316	25
	0.50	241	43
	1.00	148	65
Interferon Precursor	[IFN] (U ml^{-1})	c.p.m. per μg protein	% Inhibition
^{14}C-leucine	0	1,229	0
(2 μCi ml^{-1})	30	1,204	0
	100	1,171	3
	250	1,182	3
	500	1,165	5
	1,000	1,110	9
^{3}H-mannose	0	479	0
(5 μCi ml^{-1})	30	460	4
	100	461	4
	250	453	5
	500	446	7
	1,000	437	9
^{14}C-glucosamine	0	934	0
(2 μCi ml^{-1})	30	941	0
	100	926	1
	250	911	2
	500	873	7
	1,000	860	8
b, Infectivity			
Conditions	PFU ml^{-1}	c.p.m. ml^{-1}	PFU c.p.m.
Control	9.48×10^8	3.9×10^4	2.43×10^4
IFN-treated	9.57×10^8	3.9×10^4	2.45×10^4
c, Binding to Con A			
		Concanavalin A	
Conditions	c.p.m. added	% Retained (−)α-methyl-D-mannopyranoside	% Retained (+)α-methyl-D-mannopyranoside
Control	210,000	97 ± 2	2
IFN-treated	210,000	96 ± 2	2
TM-treated	210,000	17 ± 5	2

a, Test cultures were preincubated for 6 and 16 h with TM or IFN, respectively, before the addition of radiolabelled precursors. Cells were labelled for 24 h with leucine (2 μCi ml^{-1}), glucosamine (2 μCi ml^{-1}) and mannose (5 μCi ml^{-1}), extracted once with 10% TCA and twice with 5% TCA. The precipitate was dissolved in 0.5 M NaOH, neutralized with HCl, diluted in Aquasol (NEN) and counted by liquid scintillation spectrometry. A representative experiment is shown; however, the experiment was performed several times by different individuals with similar results. In addition, incorporation of radiolabelled precursors was monitored for 48 h with IFN, and no inhibition of incorporation was observed. *b*, Cells were pretreated with IFN (30 U ml^{-1}) for 16 h. Virus production, purification and infectivity assay are described above in the legends to the figures. *c*, The amount of ^{14}C-leucine radioactivity associated with the purified viral particles was determined, and equal number of counts were applied to gels to show that these fractions contained equivalent amounts of virus-specific proteins (see Fig. 3). The total protein in these fractions was shown to be the same by the Lowry procedure. As IFN treatment (30 U ml^{-1}) of the L-cells inhibited viral production by 10–20-fold, we had to use 10 times more IFN-treated than control cultures to obtain a similar yield of viral particles. Cells were treated with 1.0 μg ml^{-1} of TM. The binding to Con A was performed according to the procedure of Gibson *et al.*[15], except that the lectin-coupled gel beads were in suspension.

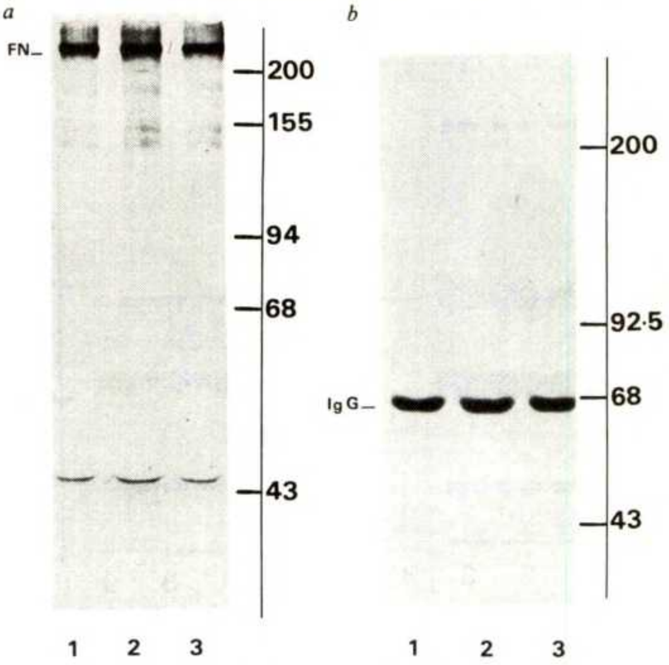

Fig. 2 Effect of IFN and TM on glycosylation of specific cellular proteins. *a*, 3T3 cells were treated with IFN and labelled with ^{14}C-D-glucosamine as described in Fig. 1 legend. FN was isolated by immunoprecipitation as described previously[3]. Lanes 1, 2 and 3 represent 0, 30 and 250 U IFN ml^{-1} respectively. *b*, Plasmacytoma cells were treated with IFN and labelled with ^{14}C-D-glucosamine as described in Fig. 1 legend. IgG was isolated by mixing 1.0 ml of the conditioned, cellular debris-free medium with 40 μl of goat anti-mouse IgG (160 μg protein; Cappel) and incubated for 60 min at room temperature. The precipitate was collected by centrifugation, and washed twice with phosphate-buffered saline. Lanes 1, 2 and 3 represent 0, 30 and 250 U ml^{-1} IFN. Similar results were obtained with IFN concentrations up to 1,000 u ml^{-1}.

However, we found that >94% of the purified viral particles, from both control and IFN-treated cultures, were specifically adsorbed to Con A as shown in Table 1*c*. As this lectin binds to mannose-containing oligosaccharides, this suggests that glycoprotein G is glycosylated in the presence of IFN. This was confirmed by direct fluorographic analysis of ^{14}C-D-glucosamine labelled viral particles (Fig. 3, lanes 4–6).

Previous studies have shown that the absence of the carbohydrate moiety caused a drastic reduction in the amount of G protein incorporated into the released VSV particles[13–15]. Because the infectivity of VSV is dependent on this protein[15–21], this was used as an indirect measure of G present on the released virions. An equivalent number of viral particles, isolated from control and IFN-treated cultures, was spread on a lawn of L-cells, incubated for 72 h at 37 °C and scored for PFU. The particles produced in the presence of 30 U ml^{-1} of IFN (the concentration used by Maheshwari *et al.*[1,5–8]) had the same specific infectivity as those produced by untreated control cultures (Table 1*b*). However, IFN drastically inhibited (10–20-fold) the production of VSV, and the viability of the IFN-treated, VSV-infected cells was not significantly compromised compared with infected cells not treated with IFN. Concentrations of IFN $\geq$150 U ml^{-1} did decrease infectivity, but had no effect on glycosylation. This finding suggests: (1) that IFN treatment does not selectively inhibit the incorporation of G into viral particles, and (2) that the primary mechanism of IFN's antiviral action is by inhibition of viral production as previously reported[22].

To demonstrate directly that the released virions are not defective in glycoprotein G, viruses were isolated from control and IFN-treated cultures and analysed by SDS-PAGE. The four viral proteins (L, G, N and M) are clearly visible in the fluorogram of ^{14}C-leucine-labelled viruses produced in either the presence or absence of IFN (Fig. 3, lanes 1–3), and the relative amounts of the four proteins are unaffected by IFN treatment. Careful inspection of the published data of

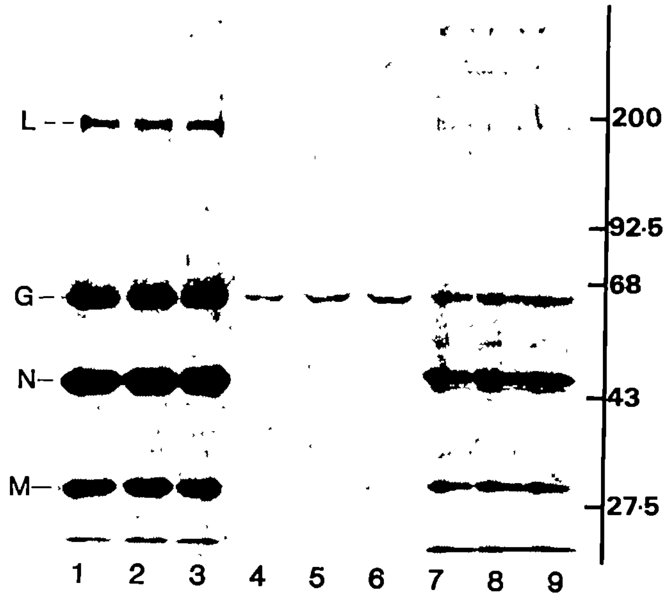

Fig. 3 Effect of IFN on protein G. L-cells were treated with IFN and metabolically labelled as described in Fig. 1 legend. To prepare purified viruses, the culture media were collected and centrifuged at 10,000*g* for 10 min to remove large cellular debris. The supernatants were centrifuged at 48,000*g* for 2 h. The pellets containing the virus were further purified by the sucrose density gradient procedure of Leavitt *et al.*[13]. The viruses were resuspended in MEM and centrifuged for 1 h at 100,000*g* to remove the sucrose. The viruses were then resuspended in MEM (without supplements) containing 100 μg ml^{-1} antifibronectin coupled to Sepharose 4B gel beads and incubated for 1 h at 5 °C on a rotatory mixer. The gel beads with the fibronectin containing membrane particles were removed by centrifugation at 10,000*g* for 10 min. The purified virus particles were stored in MEM at −70 °C, or immediately analysed by SDS-PAGE. Lanes 1, 2 and 3 represent the highly purified viral particles from cells treated with 0, 30 and 250 U ml^{-1} IFN. Glycoprotein G (lanes 4–6) was immunoprecipitated from 1 ml of a 1% Triton X-100 extract of viral particles (100 μg protein) by the addition of 50 μg of affinity-purified anti-G, and processed as described in Fig. 2 legend. Lanes 7–9 represent the crude viral particles from cells treated with 0, 30 and 250 U ml^{-1} IFN.

Maheshwari *et al.* (ref. 1, Fig. 1) shows that one likewise cannot make a strong case for a significant and specific decrease in glycoprotein G, contrary to their previous reports[6,7].

The results shown in lanes 1–3 of Fig. 3 are with viral particles that have undergone extensive purification, hence it is possible that virus with altered G protein was preferentially lost during purification. This possibility was evaluated by two different approaches. First, we determined the rate of G protein synthesis and glycosylation by pulse labelling with ^{14}C-leucine, ^{3}H-mannose or ^{3}H-glucosamine for 1 h at 3, 6 and 9 h post-infection of L-cells. Cells containing the newly synthesized viral protein were homogenized and analysed by SDS-PAGE. The polypeptide band corresponding to the G protein was cut from the gel, dissolved in 30% hydrogen peroxide and the radioactivity determined by liquid scintillation spectrometry. There was no apparent difference in the specific activity (^{14}C c.p.m./^{3}H c.p.m.) of carbohydrate precursor incorporation in the presence or absence of IFN (30–500 U ml^{-1}). The second approach involved the rapid isolation of viral particles without extensive purification. Media from VSV-infected cells, cultured in the presence or absence of IFN (30–500 U ml^{-1}), was centrifuged at 10,000*g* for 10 min, and the resulting supernatant at 100,000*g* for 30 min. The pellets containing the virus were homogenized and equal amounts of protein were electrophoresed. The fluorograms are shown in lanes 7–9 of Fig. 3. It is apparent that similar results are obtained with crude and highly purified viral preparations.

The results presented here show that IFN does not inhibit the glycosylation of asparagine-linked glycoproteins as previously reported[1]. When [2-^{3}H]mannose was used as an alternative labelling substrate for glycoproteins, similar results were obtained. Therefore, the results obtained with ^{14}C-D-glucosamine are not experimental artifacts. We conclude that the antiviral, antitumour and immunomodulatory actions of IFN are probably unrelated to glycosylation.

The present study does not agree with earlier reports by Maheshwari *et al.* claiming that IFN is an inhibitor of glycosylation[1] and that VSV released from IFN treated cells is deficient in glycoprotein G[6,7] and, as a consequence, is less infectious than virions released by untreated cells[1,5–8]. The basis for the apparent discrepancies in results are not immediately obvious as we used what we believe to be the same cells, virus and IFN as the previous investigators, and as we also used the same general procedures. Also, we were able to repeat the above results with a similar preparation of IFN obtained from Lee Biomolecular Research Laboratories. There is a major difference in experimental approach relative to viral purification; however, the apparent contradictions cannot be due to the isolation procedure as we were unable to repeat their findings even when we used less pure viral preparations. It is also important to point out that Maheshwari and Friedman[1,5–8] never studied the effect of IFN on the synthesis of host cell glycoprotein. Also, we have no information relevant to their finding that IFN inhibits the transfer of *N*-acetylglucosamine from UDP-*N*-acetylglucosamine into glycolipids[1].

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Corrigenda

In the letter 'Crystal structure of a microbial ribonuclease, RNase St' by K. T. Nakamura *et al.*, *Nature* **299**, 564–566 (1982), four residues are shown incorrectly in Table 1. In RNase St, residue 212 should be 'Q', in RNase Ms residue 32 should be 'D', in RNase Ba residue 49 should be 'K' and in RNase U_2 residue 75 should be 'D'.

In the article 'Fossil mammals and artefacts from the Middle Awash Valley, Ethiopia' by J. E. Kalb *et al.*, *Nature* **298**, 25–29 (1982), the acknowledgements section should include thanks to the Boise Fund (Oxford University) for supporting one of the authors (Dr P. Whitehead).

MATTERS ARISING

The homing mechanism of pigeons

THE review by Gould[1] misrepresents essential aspects of our knowledge about pigeon navigation. Within the limited space available, I cannot substantiate this for the whole article, but can only enumerate the most drastic shortcomings concerning my own work.

In aviary (palisade) experiments: (1) The related papers[2–4] primarily deal with recoveries which comprise sufficiently large numbers (totalling 691!) to prove statistically significant differences between many of the differently treated pigeon groups. Gould discards all these data and considers only initial bearings, although it has been explained why those data are much less reliable (ref. 4, pp. 215–216). (2) Gould's Fig. 2 is meaningless (i) because of the generally restricted value of those initial bearings, (ii) because the semi-louvred palisade used at first[3] cannot be directly compared with the open aviary (ref. 4, p. 216), (iii) because the figure mixes different release and home sites in an unbalanced way, and (iv) because it does not even coincide with the published data[2,3].

(3) Gould lumps the data of two 'glass and open lofts' and two 'wood and louvred lofts' irrespectively of the orientation of the corridors open to airflow although just this alignment has been shown to influence the pigeons' behaviour[4]. (4) Gould's site-by-site calculations of angular deviations of experimentals from controls (Table 1) conceal rather than disclose the net homeward orientation, as the relation to home is abolished. (5) "These effects" (of the hybrid aviaries) "are generally interpreted as disrupting the pigeon map sense." I wonder where Gould read this. I stressed the influence of the corridors on the preferred compass direction only, but not on homeward orientation, the part requiring a functioning 'map'.

(6) Real differences in homeward orientation have been found between pigeons from completely open or louvred aviaries and much poorer oriented pigeons from aviaries completely shielded with glass walls[4]. These findings, however, primarily concern the "small proportion" of recoveries (sample sizes 58 and 63) and thus are neglected by Gould. (7) The corridor–aviary results[4] do not contradict "the olfaction hypothesis", but only a specific version that was published at that time[5,6].

Concerning the role of olfaction: (1) Gould writes that in my experiments the nerve-sectioned birds were "slower to home" than untreated pigeons. In fact, they did not home at all[6]. (2) It is well known that anosmic pigeons are not disoriented, but that their preference for a particular compass direction persists[6–8]. When homing is concerned, however, the level of homeward orientation is crucial[6,8], and the parameters extracted by Gould (vector lengths 0.8 and 0.4, average deviation 38°) include no information on that. Homeward components of anosmic pigeons (initial bearings as well as recoveries) are drastically and significantly reduced[6,9].

(3) Gould quotes Keeton's mention of a few nasal-tube pigeons that had landed after a short flight[10], but ignores the much more conclusive 75 recoveries of anosmic pigeons (and 61 of controls) at distances ranging from 10 to 700 km reported in my paper[6]. Differences between experimentals and controls in flight directions, yet not in distances, are highly significant. There is no "contrast to the disoriented birds in other" (which?) "experiments who continue gamely flying".

(4) In the nasal-tube experiments of Keeton *et al.*[11], "no disorientation was evident". This is true, but appropriate data analysis revealed significant differences between experimentals and controls in initial homeward orientation[6], and this is the crucial point (see above). (5) Gould confuses the matter when he mixes the primary question of whether olfaction is a substantial component of pigeon homing with the secondary question of how it might be integrated into the navigational system. By considering one specific hypothesis[5] about this secondary problem, and thus many experiments merely indirectly concerned with olfaction, he creates unjustified doubts also with respect to an affirmative answering of the primary question. After recent extensive discussion of the related problems[6], this kind of review of the field is obsolete.

The examples selected may warn the reader against trusting this review. Related literature published in the meantime is quoted in ref. 12.

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IN his recent article on the homing mechanism of pigeons, Gould[1] says that when I replicated experiments with anosmic pigeons in Germany[2] no effect on initial orientation was evident, though homing speed was slower than in controls.

I would like to point out that, in my first series, pigeons made anosmic by inserting nasal tubes were homeward oriented and performed rather well in homing from a familiar site, whereas, when released from an unfamiliar site, they were randomly oriented, unlike the homeward-directed controls (the difference in homeward directedness was significant). To say that the homing speed of experimentals was slower is an overindulgent assessment of their performances; 12 out of 16 birds were lost (against 2 controls out of 18).

In my second series, both controls and experimentals underwent the section of one olfactory nerve and the plugging of one nostril (the contralateral in experimentals, the ipsilateral in controls). Despite this minimization of differences in treatment, homing performances were significantly worse in experimentals, with 61 birds out of 92 lost (against 36 controls out of 90). In this case no differences in initial orientation could be found for the simple reason that bearings of both controls and experimentals were randomly distributed. In this particular respect my experiments were inconclusive, whereas the rest of my results support the olfactory hypothesis.

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GOULD[1] writes that certain results obtained by my colleagues and myself, which support the olfactory hypothesis, "admit of simpler non-olfactory explanations". According to a first, "simpler" explanation, pigeons with nostrils plugged or olfactory nerves cut are slower in homing because of "a generalized, distracting trauma". Besides the fact that anosmia affects not only homing speed but also homing success[2–4], Gould fails to mention other important findings. (1) Homing capacity is strongly reduced in anosmic birds as compared with controls subjected to the same trauma (one nerve cut, one nostril plugged[5,6]). (2) Birds not subjected to trauma are indeed impaired in homing when prevented from smelling by nasal tubes[2,3,6], and disoriented after treatment of the olfactory mucosa with local anaesthetic, provided that smelling during transportation has been prevented[7]. (3)

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Anosmic pigeons are greatly impaired in homing from unfamiliar sites, but are not "distracted" by trauma when released from familiar ones[2,5,6,8]. Thus, anosmia affects navigation, not motivation or capacity for homing by using familiar landmarks[4].

The rotated orientation of deflector-loft birds[9-11] is attributed by Gould to the rotation of polarized light and of other visual cues rather than to that of incoming wind (another "simpler" explanation). Unfortunately, he drastically misinterprets the findings of Waldvogel and Phillips[12-14]. As these authors showed, pigeons raised in special lofts, which rotated wind and visual cues in different directions, were strongly deflected in accordance with wind-borne cues (Gould has probably got mixed up between pigeons raised in the deflector-lofts and foreign birds kept in them for a short time).

Other experiments of our group, Gould states, "cannot be repeated" or "have proved difficult to replicate". However, among the replications of our experiments performed abroad there is at least one for each kind of experiment that was successful. Fiaschi and Wagner[15] obtained positive results by applying masking odours to the beaks of pigeons, and the detour experiments of Schmidt-Koenig *et al.*[16] were successful. In fact, when one pools the bearings that were expected to deflect to the left or right in all the tries, one obtains two distributions which are different ($P < 0.005$) and rotated as predicted.

Three more of Gould's statements must be challenged. (1) It is incorrect to say that homeward orientation in anosmic pigeons has never been abolished. When releases were performed avoiding the effect of directional biases, initial orientation did not turn out to be homeward or showed a very poor homeward tendency[4,17,18]. (2) It makes no sense to say that we found that "homing speeds in the strong-odour experiment were unaffected", since untreated controls for comparing homing speeds were not used[19]. (3) It is not true that we found that "rearing birds with no olfactory experience whatsoever does not affect orientation". In the case[20] quoted by Gould, the experimentals were oriented randomly or in a direction different from that of home and controls.

Pigeon homing is a complicated, intriguing problem, but I do not feel that Gould's review contributed to its clarification. The part of his report concerning the olfactory theory appears to be more biased than one might honestly expect from the supporter of a rival hypothesis.

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GOULD REPLIES—Wallraff's unusual techniques, data analyses and conclusions have always been found difficult to interpret. My approach was to apply a more conventional analysis to such of his original data as could be extracted from his papers. As indicated in the review, this included correcting the data for release-site bias—the "correct" orientation of birds is that adopted by controls rather than the actual map bearing—and considering only that universally used measure, the vanishing bearings. Viewed in this light, Wallraff's data begin to make sense to me. Wallraff, however, prefers his own methods.

Benvenuti, in the abstract of the paper cited, states "the poor initial orientation in either controls or experimentals in many single experiments and in pooled data was an insufficient basis for evaluation of the influence of olfactory deprivation on homeward directness". In other words, there was no clear effect on initial orientation.

As indicated in the review, Papi's results generally could not be replicated by either Keeton or Schmidt-Koenig. Even a casual reading of the papers cited removes the force of Papi's present remarks—in no case do the experiments cited clearly support the olfactory hypothesis to even a mildly sceptical mind. This is not to say that odour may not play a part in homing.

My conclusion, then as now, after reviewing all the evidence, is that "the olfactory hypothesis, though initially very exciting, has lost some of its lustre as a result of these many problems. It may yet turn out to be true . . ." The interested reader should examine the original papers bearing in mind (for what they are worth) the criticisms of the various methods and conclusions outlined in my review.

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Incorrect colicin sequence

In response to an enquiry from Mr R. Parker of New Haven, Conneticut: In 1979, Dr D. H. Watson and I reported N-terminal and C-terminal amino acid sequences for colicin E1[1]. In the same paper we also described a cell-envelope-associated proteolytic activity that cleaved colicins E1, E2, E3 and K specifically to yield fragments that had either biological 'killing' activity or cell-binding properties. We suggested that such proteolysis might normally function to facilitate the transport of a colicin fragment to its target site for killing.

Early in 1980, Dr S. Luria, at the Massachusetts Institute of Technology, informed me that the N-terminal data obtained by Dr Watson were not consistent with those obtained in his laboratory, and in the summer of 1980, I circulated a note to a number of laboratories working with bacteriocins and similar proteins, informing them of our questionable data. Subsequently, direct sequencing of the colicin E1 gene[2] has confirmed that our published sequence data are erroneous. Moreover, although cell-envelope-mediated proteolysis of colicins of the kind described in our report has been demonstrated by several laboratories, its specificity and role in normal colicin function are not proven by our experiments.

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Matters Arising

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BOOK REVIEWS

Fools and pedants

Fred Dainton

Universities, Politicians and Bureaucrats: Europe and the United States. Edited by Hans Daalder and Edward Shils. Pp.510. ISBN 0-521-23673-8. (Cambridge University Press: 1982.) £37.50, $74.50.

FIFTEEN years ago student unrest was widespread. Those of us who experienced it think of that period as a bygone age and speculate what those students, now in their mid-30s, are doing. But that is not the only question to be asked. More important are: Why was the movement in the United Kingdom so mild relative to those in Germany, France and the United States? Was it because it was managed better at the university level, that vice-chancellors are better than rectors and presidents at student relationships? Was it that the internal structures of the British universities and their interdigitation with central government were more adaptable, better able to absorb the assault on their values and to turn the anger into something more productive? And perhaps of greatest importance: What did this unrest imply for the prospects of universities in the future?

In so far as Britain is concerned the answer to at least one aspect of the last question is plain. As a former chairman of the University Grants Committee cast in the role of securing resources for universities, I can assert categorically that nothing made my task harder than the inanities and the behavioural extremes of those tiny groups of militant students and their unwillingness to allow free speech by others. They damaged the student image and with it the cause of university education.

Many books have been written about the causes of student unrest and the forms it took. By and large the techniques of "direct action" — the "sit-in", the attempts to give it legitimacy through the "general assembly", the trappings of a participatory democracy and so on — were common to all countries. So too were the characteristics of the activists, being drawn largely from the upper rather than the lower socio-economic groups and prone to fragment into doctrinally distinct groups — Maoists, Anarchists, Trotskyites, orthodox communists and those flirting with Laingian psychology or the drug subculture. The immediate causes seem to have differed from country to country; protest against the Vietnam war in the United States, nuclear power in Japan, the military–industrial complex generally.

This book is quite different. It is concerned principally with the way in which the universities and the governments in the United States and Europe reacted to student unrest. It was commissioned in 1974 by the International Council on the Future of the University (ICFU), which had grown out of the concern of a group of individual academics who met in 1970. They were convinced that a crisis existed in universities and that some fundamental academic and social values were threatened because, at least in the view of some of ICFU, governments and universities in some countries were accommodating to the unrest to extents which compromised those values. ICFU therefore asked Hans Daalder, Professor of Political Science at the University of Leyden, to bring together a group of scholars of high reputation, each of whom would be able to write about a particular country with scholarly objectivity.

The result of the labours of this group, mainly political scientists or sociologists, together with a lawyer and an economist, is a series of interesting essays concluded by a masterly, succinct and lucid survey by Daalder himself. There is no anger; just calm and judicial appraisal of the evidence after it had been subjected to the scrutiny of real professionals. Once started on it, I found the book extremely difficult to put down. Even setting aside what I acknowledge must be a deep personal interest in the subject, I believe that it would make enjoyable and profitable reading for anyone who cares about universities and their role in society. Indeed, I would recommend it to all who work in universities whether as administrators or as teachers, scholars and researchers, and to bureaucrats and politicians whose decisions have such a powerful influence on universities, especially in Europe. Daalder apologizes for the delay in the publication of these essays, but, in my view, that delay is a positive advantage. Not only has it allowed the writers a better perspective, the making of judgements in a more dispassionate manner than would have been possible when the phenomena being examined were also being experienced, but also the book appears at a time when the world recession has caused a reordering of social priorities and, for the students, of their personal priorities. The time has now come, as Rutherford said in the 1930s, when "We have less money, we must think harder".

The reaction in British universities to student revolt went through several phases. First there was the shock and hurt felt by academics that some students distrusted them and did not reciprocate the proffered friendship of the teacher. Then came annoyance at the disruption of work followed by resolve to identify and remove any genuine causes of grievance. Over the ensuing years adjustments were made at many levels within the universities but no vital principles were surrendered. Students were brought into governance and, like the professors, some made useful contributions and some did not; their verbosity was and remained in inverse relationship to the significance of their utterances. The essential academic freedoms of selection and assessment of students, of the hiring, firing and promotion of academic staff and of the control of the curriculum and how it should be presented were reserved as the exclusive responsibilities of the dons. Government did not intervene because it had no *locus standi* under the charter and statutes of each university and the most that it could have done would have been to persuade the Queen to revoke the Royal Charter or to instruct the University Grants Committee to withhold grants from a particular university. Either of these acts would have taken far too long to bring about and would have damaged the interests of the vast majority of students who were going about their business in the way that students have always done.

How very different was the situation in Europe, in some countries of which, unlike Britain, many universities were and are subject to special laws and professors were officials whose appointments were ultimately in the hands of the state. The disruption was greater, the student protest more violent and destructive, and there were many justifiable complaints about the manner in which many universities were run, which was more appropriate to the nineteenth century and quite unable to meet modern needs. In essence, professors were all powerful, running their institutes like private baronies; their assistants had little status and little influence in shaping the policies of either the institute to which they belonged or the university of which it formed a part. Because they did not share in this responsibility they felt no compelling reason to show loyalty to the institute or to the university when the troubles began. With no limitations on the number of students entering, and with very long courses before graduation, student numbers were high and teaching was mainly didactic, with the formal lecture to extremely large audiences the predominant mode. There was little small-group teaching and virtually no tutorials and, therefore, little personal contact with the full professors until the student proceeded to the level of serious research and the preparation of a thesis. Reforms were certainly overdue in Germany, France, Italy, Belgium and Holland. These reforms seemed beyond the imaginative power of the academics, at least during crisis, and even if the reforms could have been devised they would have been likely to be beyond the legal power of the university to implement. Therefore the state had to intervene. The forms that this intervention took in different countries are described and assessed in the book.

The overriding impression these accounts give is one of general ineptness on the part of politicians and bureaucrats; in matters of university governance they overreacted and overshot the mark by a large margin. They did so in part because they themselves were unsure of what universities should be and in part because of a rather pervasive mood that participatory democracy was a recipe for harmony in all orgaizations. A typical result was the imposition of the *Dreigruppenprinzip* on universities by which the governing body was made equally tripartite between elected academics, elected non-academic staff and elected students. The result was predictable; interminable and inconclusive meetings and the obfuscation of the basic truth that university education, whether vocational or non-vocational, is a partnership between the old and experienced and the young and inexperienced in which the former put their knowledge and experience at the disposal of the latter in a way which draws out their talents of intellect and personality as well as providing mental discipline, knowledge and skills. There is a memorable passage in one of A.N. Whitehead's lectures delivered 55 years ago:

> Youth is imaginative, and if the imagination be strengthened by discipline, this energy of imagination can in great measure be preserved through life. The tragedy of the world is that those who are imaginative have but slight experience, and those who are experienced have feeble imaginations. Fools act on imagination without knowledge, pedants act on knowledge without imagination. The task of a university is to weld together imagination and experience.

Readers of this book will classify the actors — the professors, the politicians and the bureaucrats — in their own way. They will not always agree with the perceptive comments on this brief but exciting episode in university history, but they will find much food for thought.

Sir Frederick Dainton is Chancellor of the University of Sheffield.

Bioenergetics: is there any demand for quantum mechanics?

Terrell L. Hill

Biology and Quantum Mechanics. By A.S. Davydov. Pp.229. ISBN 0-08-026392-5. (Pergamon: 1982.) $45, £22.50.

AT THE end of the introductory chapter, A.S. Davydov states the theme of his book:

> Undoubtedly the effective use of the chemical energy from food products by living organisms for muscle contraction, the production of concentration gradients, the conduction of nerve impulses, protein synthesis, etc., is associated with the excitation of special weakly relaxing degrees of freedom.

Davydov is referring here to excitons and solitons, especially in one-dimensional molecular systems (for example, the α-helix). If, however, he had used "conceivably" in the above quotation in place of "undoubtedly", he would have been on much firmer ground.

As it stands, most readers with some understanding of modern bioenergetics will be put off by some of the claims in the preface and first chapter. This is a pity because most of the book is, in fact, devoted to a review of assorted subjects in molecular biophysics and bioenergetics. Such a review could prove quite useful in providing a first glimpse of this field for a physical scientist. It is notoriously difficult for many physicists, especially theoreticians, to acquire the necessary background and perspective in order to move into biological research; a guide written by a distinguished physicist ought to ease the pain.

The potential reader should be aware, however, that the discussion of almost every individual topic stops well short of the present state of knowledge and understanding at the molecular level. The treatment of most subjects covered here is roughly five years out of date, that of muscle contraction models and theories even more so. A physical scientist who begins with this book would do well to turn next to the second edition of Lubert Stryer's *Biochemistry* (W.H. Freeman, 1981) to obtain a more thorough and current treatment of most of these topics.

To return to the initial criticism: conventional kinetic, thermodynamic and statistical mechanical concepts provide a completely adequate theoretical foundation, at the molecular level, for the understanding of all of the special biological topics treated in this book. This foundation has to be supplemented, of course, by still incomplete mechanistic and structural details in each individual case. It is not necessary to invoke any relatively exotic quantum mechanical explanations, as the author does (for example, for muscle contraction). This is certainly not to say that such approaches are untenable: rather, elementary and straightforward explanations should be retained and tested until there is evidence that more complicated theories are a necessity. There is no such need in bioenergetics at the present time.

Terrell L. Hill is at the National Institutes of Health, Bethesda, Maryland.

A class apart

William Hodos

Brain and Intelligence in Vertebrates. By E.M. Macphail. Pp.423. Hbk ISBN 0-19-854550-9; pbk ISBN 0-19-854551-7. (Oxford University Press: 1982.) Hbk £20, $29.50; pbk £10.95, $14.95.

THE histories of comparative studies of brain anatomy and of intelligence in vertebrates have followed parallel, but for the most part independent courses during the past century. Each began with anecdote, followed by a slow accumulation of hard facts. The past two decades have seen a dramatic increase in the amount of data generated in both disciplines and breaks with theoretical tradition.

Euan Macphail's book is an attempt to achieve a fusion of these two fields. Although written with considerable clarity, much of the book does not lend itself to effortless reading. Nonetheless, the exertions of the reader will be amply rewarded by an understanding of the salient issues in the brain–intelligence field and by an awareness of the fallacies, gaps in knowledge and intellectual pitfalls that have bedevilled it since its inception.

Apart from the introductory and concluding sections, each chapter is devoted to a single class of vertebrate and has the same general organization. First, the evolutionary history of the class is briefly reviewed followed by a short account of the taxonomic organization within the class. Next, the author presents a description of the anatomy of the forebrain of some individual species characteristic of the class, before turning to a critical survey of the various types of learning procedures that have been attempted with members of the class. Each chapter ends with a summary and conclusions.

The two introductory chapters discuss the measurement of intelligence in animals and human beings, offer a classification of learning tasks, summarize the evolution and classification of vertebrates and review the literature on general morphological brain indices. The two concluding chapters, which deal with the relationship between language and intelligence in human beings and the evolution of the brain and intelligence, include an analysis and critique of the studies of language and communication in non-human animals and their relationship to human and animal intelligence.

This final section also contains the author's evaluation of the current state of knowledge of comparative studies of the brain and intelligence, which he characterizes in two working hypotheses to be tested by future research. The first is that, with the exception of human beings, neither qualitative nor quantitative differences in intelligence exist between vertebrate species; the second, that the capacity for language so affects the human's approach to problems of all kinds that the result is a qualitative difference in intelligence between humans and non-human vertebrates. Moreover, the influence of language is so pervasive in human reasoning that one may be unable to assess the role of non-linguistic reasoning in humans for comparison with non-human animal intelligence. These hypotheses are refreshing departures from conventional wisdom and will be the basis for considerable discussion.

While there is much to commend in the book, the treatment of certain topics falls short of perfection. For example, the only region of the brain that is discussed consistently is the forebrain and then usually only the telencephalon. Macphail justifies this emphasis by pointing out that it is this region that appears to undergo the greatest growth and development in evolution. Although his point is well taken, critics could argue that the telencephalon does not function in isolation and has important connections with the diencephalon and mesencephalon; moreover, to ignore the role of the mesencephalon in processing information about the spatial properties of the external environment may be to disregard an important aspect of the brain–intelligence problem.

Another area that may disappoint some readers is Macphail's limitation of his account to certain types of formal laboratory studies of learning. Phenomena such as tool-use and learning by imitation are not discussed, the vast ethological literature and its implications for assessing the nature of animal intelligence being specifically excluded. Macphail makes a case for the latter omission, but I suspect that readers with an ethological bent may not be fully convinced.

Such disappointment as the reader might feel will nonetheless be soothed by the wealth of well-organized information, original interpretations and challenging ideas presented in this volume. I recommend it highly to anyone with a serious interest in the brain and intelligence. ☐

William Hodos is a Professor of Psychology at the University of Maryland, College Park.

View of Kew from the inside

S.M. Walters

Kew: Gardens for Science and Pleasure. Edited by F.N. Hepper. Pp.195. ISBN 0-11-241181-9. (HMSO/Stemmer House: 1982.) £9.95, $24.95.

THIS handsome and lavishly illustrated book consists of a series of essays by sixteen past and present members of the staff of the Royal Botanic Gardens under the editorship of Nigel Hepper, himself a senior taxonomist in the Kew herbarium. The subject-matter covers all three parts of the Kew empire, namely: the public gardens themselves; the herbarium and other research institutions not normally visited by the public; and Wakehurst Place, the magnificent "satellite" garden in Sussex, which became part of Kew in 1965.

The introduction explains that the essays "are intended to convey a broad view of Kew's history and the horticultural and scientific work that have given the Gardens their world-wide reputation". Ray Desmond, formerly Chief Librarian at Kew, contributes the first chapter, which sketches the history of the Royal Gardens, and the remaining essays are grouped into three sections: "Gardens for Pleasure" (3 chapters); "Living Botanical Collections" (9 chapters); and "Botanical Research" (7 chapters). The decision to integrate Wakehurst with the "old Kew" by devoting chapters to the new garden in each of the main sections, though logical, has not been entirely successful; in particular, the description of the memorial garden to Sir Henry Price, last private owner of Wakehurst, seems out of place in the first section.

Tropical splendour in Surrey — interior of the Palm House at Kew (1852).

Illustrated London News

A welcome feature of the book is the treatment of aspects of Kew as a botanical research establishment. Chapter 17, devoted to multidisciplinary studies of *Crocus,* represents a brave attempt by no fewer than five staff members to explain at a popular level mysteries such as cytotaxonomy: fortunately, the genus lends itself to attractive colour photography! Another important chapter deals with Kew's new, explicit conservation role, the most urgent of all its tasks in face of the threat to the world's flora.

Reading the essays produces a strong impression that the authors are all loyal Kew people. A measure of how parochial their view is can be obtained by looking up other botanical gardens in the index: for example, Edinburgh, by any standards one of the world's most important scientific gardens, gets only a single, oblique reference, on page 31. Even in those few areas where the outside world has dared to question the morality of Kew's activities — such as the manner of acquisition of stocks of rubber plants in South America a century ago — the loyal Kew man says only (p.131): ". . . popular accounts indicating that Wickham dishonestly smuggled out the *Hevea* seeds are unfair both to the collector and to Kew". At least a strong antidote to such sycophantic writing is available (if in very extreme form) in the recent study by Lucile Brockway (1979) cited in the select bibliography at the back of the volume. Kew still awaits a good, definitive study, preferably by a reasonably sympathetic and well-informed outsider, who can ask questions about policy and aims such as are hardly touched on in this work, even in the interesting final essay on "Kew in the Future" contributed by the retired Director, Professor Brenan.

The world of book publishing presents increasingly baffling questions to the layman. Just at the time when we are told that Her Majesty's Stationery Office will no longer subsidize official publications of any kind, that same Office publishes such a lavish, popular book as this at a price which implies a large subsidy or great optimism and an enormous print run. Whatever the explanation, we can only express our appreciation, and wish the venture success.

S.M. Walters is Director of the University Botanic Garden, Cambridge, and author of The Shaping of Cambridge Botany *(Cambridge University Press, 1981).*

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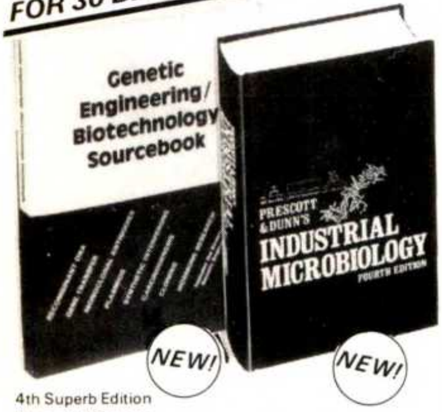

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A few fruits from a large orchard

John B. Furness

Biology of Serotonergic Transmission. Edited by Neville N. Osborne. Pp.522. ISBN 0-471-10032-3. (Wiley: 1982.) £30, $69.

IN MAN, serotonin (5-hydroxytryptamine) nerves have been implicated in the control of various functions, including sleep, temperature regulation, pain and the secretion of pituitary hormones. At the other extreme, serotonin nerves in annelids and molluscs have been extensively studied because their prominent cell bodies can be identified by size and position in any individual animal. Serotonin is also found in other invertebrates, and, in the various vertebrate classes, is present in both the CNS and peripheral (intestinal) nerves.

The declared purpose of this book is to provide an up-to-date report of the state of serotonin research in the 1980s and, to a large extent, the various authors have done well by the topics covered in their individual chapters. However, in that the book only partly covers the field, Dr Osborne might well have referred his readers to other books and reviews — for example to the massive five-part work *Serotonin in Health and Disease*, edited by W.B. Essman and published by Spectrum Publications in 1978–1979.

Broadly, the articles in the present volume can be placed in one of two groups — those dealing with the (mainly biochemical) properties of serotonin neurones and those dealing with serotonin neurones in particular organs, species or life stages.

In the first group there is excellent coverage of the biosynthesis of serotonin, its storage and release, axonal transport, turnover, and uptake into axons and storage vesicles. Except for the chapter on axonal transport, which also deals with molluscs and makes occasional comparisons with platelets, these contributions are concerned with the properties of serotonin neurones in the CNS of mammals, principally the rat. This imbalance is only partly redressed in the final chapter of the book where some of the biochemical characteristics of serotonin neurones in gastropods are described.

Included in the second group is a succinct but commendably clear summary of the arrangement within the mammalian CNS of serotonin nerve cells and their processes. Another chapter documents the use of radioactive ligand-binding studies to distinguish between 5-HT_1 and 5-HT_2 receptor types in the CNS. These studies may eventually provide a basis for interpreting differences in the behavioural effects of drugs acting at serotonin receptors. Possible roles of 5-HT in the mammalian central nervous system are dealt within a group of three further contributions; this is one of the weak parts of the book because so much is left out. The first summarizes the putative roles of serotonin but is disappointingly brief. And only two aspects of serotonin's central roles are dealt with at length in the other two chapters, namely serotonin and learning, and serotonin involvement in dyskinesias (the latter subject also appears at the end of a separate article on serotonin neurotoxins).

Serotonin nerves of the mammalian gastrointestinal tract (with some reference to other classes of animal) are reviewed next. As the existence of serotonin nerves in the gastrointestinal tract has been difficult to establish, the author gives proper emphasis to evaluation of the evidence for their presence. Another subject of some controversy, namely the proposed presence of serotonin neurones in the vertebrate retina, is covered in the following contribution. Finally, two chapters give lucid accounts of current research on serotonin neurones in leeches and gastropods.

Perhaps more than anything else in this field, a comprehensive and critical review of the pharmacology of serotonin receptors is needed. Yet in the only chapter explicitly dealing with drugs acting on serotonergic neuronal systems, a mere two pages and a table are given to a disjointed discussion of the subject. Not only is there a failure to do justice to receptor classification, but the very real problems of the additional actions of serotonin antagonists are overlooked; for example, the agonist actions of methysergide and LSD are not mentioned, nor is the antihistaminic action of cyproheptadine. Most of the "non-specific" actions of other drugs acting on serotonin systems are also ignored.

Patchy coverage and lack of cohesiveness are common in edited works of this sort. But here they have reached such a pitch — inhibitors of the synthesis of serotonin are dealt with in four separate articles without cross-referencing, for example — that most of the contributions might just as well be in review journals. In mitigation there are some excellent chapters which are valuable works of reference in their own right. However for only a part of its potential audience will these chapters justify purchase of the entire book. ☐

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Paper Fuller

Macmillan, New York/Collier Macmillan have issued a paperback edition of R. Buckminster Fuller's *Synergetics: Explorations in the Geometry of Thinking,* first published in 1975. The new edition incorporates revised illustrations, and costs $12.95, £9.95.

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nature

25 November 1982

Coming out of the long tunnel

British universities have weathered enforced economy better than many feared. The return to normalcy may be more testing.

In the two years since the British government told its dependent universities that they would have to share in a general reduction of public expenditure from which only the armed services and the police were excepted, many British universities have learned some unacademic tricks. Half-way through the adjustment period, due to end with 1983-84, most universities have been able to reduce their payrolls by natural wastage and because of the attractions to those older than 50 of the early retirement schemes sanctioned by the Treasury. Only when the dust has settled will it be known whether the academics who have left include an undue proportion of those who can be least spared — and whether the cost of paying for these early pensions, a charge on future budgets, will be supportable. At the same time, many universities have made deals with industry or commercial concerns that help both to maintain the scale on which research is undertaken and to shed some of the cost of the tenured payroll. Again, it is too soon to know how seriously these developments will compromise the quality of teaching — or the impartiality of the universities concerned. But especially because the research councils have also done their best to rally round, and with the prospect of extra government money for the appointment of younger people to university faculties, it is natural that academics should be looking forward to the ending of this long period of deprivation.

That temptation should be resisted. The months between now and September 1984 could yet bring trouble. The announcement last week that the University of Aston (in Birmingham) is planning to fire tenured academics is a sign that the university has exhausted the supply of academics willing to settle for early retirement. Other places could yet find themselves in the same boat. It is also likely that the universities that have been unable or unwilling to comply with the new (reduced) targets for student numbers put out in June 1981 by the University Grants Committee will find themselves in further trouble before the present adjustment period is over. The British government is unlikely to condone such cheekiness, while the grants committee will probably find itself having to punish the transgressors on the grounds that they are making an inequitable claim on national resources. Precisely how these issues are resolved will depend to some extent on the appointment of the next chairman of the grants committee. Quite apart from the administration of a shrunken budget, the successor to Dr Edward Parkes will have to shoulder two less tangible burdens — keeping up the spirits of British academics and making good the erosion of academic standards in British universities.

Structure

Grappling with these problems would be easier if the structural problems that afflict the British university system had been solved. On past form, however, that is unlikely without external intervention. William Waldegrave, the minister at the Department of Education and Science with responsibility for higher education, was right last week to remind his audience at Westfield College, London, that British universities and academics should have been worrying about the way in which the system is run at least since 1977, when Mrs Shirley Williams, then in charge of the Department of Education and Science, sympathetically spelled out her version of what was wrong. Broadly speaking, British universities ignored or denied that warning; they have since found themselves coerced by the government instead. The intelligence that even the previous Labour government was alarmed at the rising cost of educating students from overseas evoked from British academics an opinion that to change the previous practice of equal access would be illiberal. The Labour government's imaginative offer of a system of scholarships for overseas students was not seriously discussed, with the predictable result that the Labour government's successor, the present administration, impatiently instructed British universities that fees should be paid by students from overseas. Much the same is likely to happen if British universities now continue to pretend that persisting anomalies in higher education are no concern of theirs: the government (this or the next) will intervene again.

Support

The cost of supporting students in higher education is plainly bound to be a running sore in the years ahead. Present arrangements require that local authorities should pay the tuition fees of all students accepted for places in higher education and provide them with maintenance grants as well. Local authorities recover almost all the cost of these subventions from the central government, which is why the government complains that higher education (not just universities but polytechnics and colleges of further education) has an open claim on public funds. This feature of the present arrangements for educating young people in Britain is the reason why the University Grant Committee was compelled, in June 1981, to instruct every British university that, by 1983-84, the numbers of home students should not exceed some arbitrary quota. Universities are well within their rights to argue that the consequences are often uneconomic. Universities would also be technically within their rights if they complained that the government has been slow to set up a mechanism for regulating the cost of other sectors of higher education. They will, however, be unwise if they continue to insist that their decisions on which students they are prepared to teach are financially binding on the central government; that position could easily be turned if the government decided that students would automatically be provided with maintenance grants calculated only to be enough to allow them to attend their neighbourhood university, but with a loan scheme allowing them to go further afield if they have the chance. That, or a system of competitive scholarships for potentially able students, is likely to be forced on the British universities if they find nothing better.

The structural problem occasioned by the uniformity of the British university system, and its ideological separation from the rest of British higher education, also needs attention. As is well known, British universities seek to be like Oxbridge but will occasionally settle for a different kind of self-image if it happens to be something equally distinctive, that of Imperial College, London, for example. Farcically, all British universities count themselves at least one rung higher on the ladder of intellectual opportunity than the polytechnics. That conceit, however, is mistaken. Many polytechnics provide their students with a better education (a more employable skill) than some universities. Some polytechnics are more distinguished than some universities at research. So is it wise of the universities to continue to insist that the rest of higher education is untouchable? That difficulty would

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become especially hazardous for the weaker universities if, as seems probable, the British government finds a way of ridding the universities of the quota system under which they now have to labour, for then the weak among them would be known not merely in common-room gossip but from their success (or the opposite) in attracting students.

Another abiding problem of British higher education which academics stolidly refuse to contemplate is educational. British universities, insistent as they are on the equation between academic excellence and academic freedom, and used to equating freedom with the right to do what they have always done, pay very little attention to the way in which they educate their students. Prepared though they are to go to the barricades whenever the grants committee should suggest that one or other of them should abandon its degree course in agriculture or Portuguese, their willingness to delegate the selection of the students whom they will teach to a computer centre in Cheltenham is a proof that they are only half-serious. If British universities hope to survive, they must set out to choose their own students by their own devices, snapping their fingers at the cumbersome apparatus of school-leaving examinations but saying instead that they will provide a higher education for sufficiently able if ill-prepared students even if that means teaching them for four years and not just three. The government would not welcome such a development, but would not be qualified to resist.

But are British universities not autonomous institutions, whose academics collectively decide what kinds of places they should be? That is the theory, but no longer the practice. In an ideal world, or even in the United States, universities conscious that their reputations leave something to be desired would think of strengthening their claims on student and academic attention by offering good teachers attractive terms of service. In Britain, there is instead a nationally agreed scale of salaries for academics from which Oxbridge is to some extent exempt. The result is that even when some provincial university is able to create a superb department in some field, the advantage is bound to be short-lived; the people concerned will eventually be hired away to a more prestigious or congenial place. If on the other hand some university should model itself on some place that is not Oxbridge but, say, the University of Aachen or the Massachusetts Institute of Technology, it would quickly find itself regarded by its peers as a kind of jumped-up polytechnic — and would equally be prevented from keeping its academics by the uniformity of the terms of service under which it (and they) labour.

The agenda accumulating for the next chairman of the University Grants Committee is therefore formidable: how to share out a government subvention while providing those who depend on it with an incentive towards diversity, how to educate most students more broadly without sacrificing the service now provided by those universities that are already excellent, and how to make a bridge between universities and other higher education establishments without humiliating either. There follows from this a simple recipe:

- Pensioner universities should be compelled towards autonomy, told in advance what their budgets will be for as many years ahead as can be managed but then subjected to an intellectual rather than procedural review of their academic attainments.
- Universities should be reminded (some have forgotten) that the reason for their existence is the education of the young; the opportunity for research follows from that, and is not capable of being institutionalized by itself. Getting hold of suitable entrants to undergraduate courses should therefore be central to most universities' concerns (in which case the abandonment of school-leaving examinations and the disbandment of UCCA will come naturally). Teaching elementary courses (and worrying about how well they are doing) will naturally frighten British academics (in which cases they should be fired).
- Making bridges with the rest of higher education should be relatively simple: why should not the University Grants Committee provide support for the infrastructure of good research whenever it may be carried out?

Biting off others' tongues

The UK Medical Research Council seems to be irrationally sensitive about a planned clinical trial.

The blank space on page 310 is intended to be spectacular. Earlier this week, the space was occupied by a reasonable letter replying to an earlier piece of correspondence from Dr Arthur Wynn (see *Nature* 11 November, p.102) raising doubts about the British Medical Research Council's plan to carry out a controlled study of the utility of folic acid as a dietary supplement in the prevention of recurring embryonic neural tube defects, usually manifested as spina bifida in human births. After the page concerned had been readied for printing, the author of the letter asked that it should be withdrawn on the grounds that its publication would acutely embarrass the Medical Research Council, but for reasons that he did not fully understand. As it happens, the same disputed page of *Nature* had earlier on the same day been adorned with yet another item of correspondence on the same subject arguing that the disputed trials had needlessly been delayed for two years, during which time it had become apparent that the balance between the risks of folic acid supplements, and the benefits thereof, had shifted towards the latter. That letter had also been withdrawn, again at the request of the Medical Research Council.

Objectively, there should be no problem about the plan to mount a controlled study in Britain of the influence of folic acid on the incidence of neural tube defects (spina bifida) in human births. Two years ago, the UK Department of Health seems to have taken that view, calling a meeting to plan how the trial should be carried out. Delay has allowed three categories of ethical concerns to flourish — protests that if there is evidence that folic acid will prevent spina bifida, it is unfair to deny the mothers in the control group the appropriate supplement of their diet, the claim that unnaturally large amounts of folic acid in the diet may be harmful (shades of fluoride in the drinking water) and legalistic but unrealistic worries (in a country in which abortion is permissible but not entirely free) that for a mother to consent to membership of the control group denied folic acid supplement would be to infringe the rights of a child unborn or even not conceived.

None of these arguments is absolute. The case that folic acid will prevent recurrences of spina bifida is strong but not overwhelming; it would be more persuasive if there were a convincing tale to tell of how this supplement, rather than some other, were effective. The chance that folic acid might be harmful would be more persuasive if the chemical were cheaper. The challenge to the rights of the unborn child is philosophically powerful but only to the extent that putative parents sense that unborn children could be cured of putative spina bifida by a suitable dose of folic acid.

Resolving such issues patently requires nothing less than what the Department of Health had in mind two years ago — a controlled trial set up in such a way that it could be abandoned quickly. The Medical Research Council should resolve on such a course when it meets next, on 7 December. It should not be deterred by what the politicians have to say.

Meanwhile, innocent bystanders should ask themselves why *Nature* should be printing several square inches of empty space, and why the authors of the words that would ordinarily have filled them have at the last minute been frightened away from publication. In each case, the groups concerned have said that the pressure came from the Medical Research Council. By all accounts, it would be entirely unworthy to suppose that any component of that pressure is concerned with future research grants or their availability. But can it really be sensible that a research council already sufficiently entangled with muddy and muddled political issues beyond its competence should also seek (and successfully) to suppress what its own people have to say, if not openly in its defence then in the cause of helping the understanding of a tendentious issue?

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Biotechnology patent challenged

Ex-colleague seeks share of the credit

Washington

Dr Robert Helling will formally press a claim of co-inventorship of the Cohen–Boyer genetic engineering patents (see *Nature* 11 November, p.95). Helling, now at the University of Michigan, worked with Dr Herbert Boyer at the University of California, San Francisco, during a sabbatical in 1972–73 and co-authored the 1973 paper in *Proceedings of the National Academy of Sciences* that forms the basis of the patents. He has consistently refused to sign a disclaimer of co-inventorship as requested by Stanford University and the University of California, but decided only last week to press his claim actively.

The decision seems likely to delay further the issuing of the second Cohen–Boyer patent, at present in limbo after the patent office tentatively rejected it, in part because of the unresolved role of Helling. It may also increase pressure for a re-examination of the first patent, issued in December 1980.

The decision last week came after the University of Michigan agreed to take up the case on Helling's behalf. James Dautremont, the university's intellectual property counsel, said the matter would be discussed with both the patent office and the two universities involved "in a completely cooperative effort to determine where the legally proper inventorship lies".

It is not clear whether Helling or the University of Michigan has a financial stake in the outcome. Helling did sign a patent agreement with the University of California covering his term there that appears to assign any patents to that university; if that is so, all he could gain would be the recognition of having his name added to the landmark patents. On the other hand, if it turns out that he was covered by the University of Michigan's patent regulations at the time, he would divide his share of the royalties equally with the University of Michigan.

In the response of 1 November to the patent office's proposed rejection of the second patent, the attorney for Stanford and the University of California argued that Helling's failure either to claim or to disclaim co-inventorship in the eight years since the patents were filed should not be permitted to hold up the patent. But Dautremont counters that "inventorship can be challenged or modified as appropriate according to the facts at any time". The question, he says, is whether "Professor Helling in fact made a significant contribution to the invention as recited in the claims of the patent. I'm sure that will become clear from written records, lab notebooks, and so on".

Dautremont emphasized that "we're not in a fight at all" and said he expected the matter to be resolved "amicably". And he suggested that it would be in the best interests of Stanford University and the University of California to add Helling's name to the patent if the facts warrant it in order to remove a "cloud over this issue".

Stephen Budiansky

Strike response to French reform

The plans of M. Alain Savary, French minister of national education, to set in motion what he described in early October as a "global reform" of the universities and their prestigious sisters, the *grandes écoles,* seem to be meeting with an equally global resistance.

The university trade union SNE-Sup (Syndicat National de l'Education Supérieure) has this week, therefore, called a two-day national strike, demanding that the reform be given "new life"; while the *grandes écoles,* engineering schools well represented in the government administration and traditionally regarded as providing the best education in France, have been applying their own behind-the-scenes pressure to ensure that real life does not affect them. Given M. Savary's gentlemanly approach to politics, the result may be something closely approaching the *status quo.*

Meanwhile, the water is being muddied by the ministry, which appears reluctant to reveal exactly what the minister has in mind. First, there is the unpublished — but circulating — "Jeantet report", described by conservatives as the work of a "radical dreamer". It foresees the absorption of some *grandes écoles* by the universities, and attacks élitism. It is the result of M. Claude Jeantet's questionnaire to the universities, *grandes écoles* and other institutions and was intended to form the basis of the reform, but it now it seems the minister is distancing himself from it.

Then there are three other texts, all published within two weeks in October, which purport to outline the minister's views. Two of these are speeches by Savary himself, and one — the last — is an "information note" from the ministry. All are partly contradictory. They appear to indicate an evolution towards conservatism; but the information note fails to touch on many key issues — such as research, or reform of higher degrees — and leaves room for the reform to be radical in some respects. (For example, universities are defined as including "schools" — by implication the *grandes écoles* — but *grandes écoles* are also defined outside the universities; thus *grandes écoles* directors are asking themselves which kind of school their own will be.)

As if further to confuse the issue, the director-general for higher education and research at Savary's ministry, the newly-appointed and activist M. Jean-Jacques Payan, said last week that he had been taken fully into Savary's confidence over the final shape of the reform and that it will reveal "many new things" when it is published, he predicts, on 10 December. For example, he says, the reform will allow

IIASA struggles on in hope

The International Institute of Applied Systems Analysis (IIASA), the East-West systems analysis centre in Vienna, has had a reprieve from the United States, and a partial reprieve from Britain.

In both countries, the member institutes of IIASA — the National Academy of Sciences in the United States and the Royal Society in the United Kingdom — had withdrawn as of 1 January 1983, and the problem was to find new members that could pay the subscriptions for IIASA's total budget of around £5 million (in 1981). Now the American Academy of Arts and Sciences has agreed to take over the US membership, raising the money needed from private foundations and industry.

In Britain, the situation is less clear. The Fellowship of Engineering, a body with few funds of its own, is debating whether to join IIASA, but it will have to find the whole subscription outside — either in industry or in government departments other than the Department of Environment, which originally paid the bill and whose lack of interest in IIASA led to the Royal Society's withdrawal.

Meanwhile, IIASA — crossing its corporate fingers — has drawn up its research plans for 1983. Project titles include: patterns of economic structural change and industrial adjustment; national agricultural policies; energy development, economy, and investments; structural change in the forest sector; institutions and environmental policies; population — ageing and changing lifestyles; integrated regional and urban development; adaptation and optimization; and negotiations and interactive decision making.

Robert Walgate

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all universities to teach engineering, and grant *Diplômes d'ingénieur,* previously the jealously guarded preserve of the *grandes écoles,* and not previewed in any ministry text so far.

The next step, however, is the presentation of a near-final text to the advisory Conseil National de l'Education Supérieure et de la Recherche (CNESER, the national council for higher education and research) which was scheduled for Thursday 25 November — the first day of the planned SNE-Sup strike. However, the unions have requested that the CNESER meeting be postponed, and on Monday a new date had not yet been arranged. CNESER is reported to be very closely divided between radicals and conservatives, and may vote for a text close to Jeantet — which would set the cat among the *grandes écoles* pigeons, unless Savary were entirely to ignore CNESER's advice — something that would be politically inadvisable. CNESER, in fact, along with M. Payan himself — who is *ex officio* president of CNESER — may be the main element of volatility in a situation long since frozen by the traditional conflicts between the *grandes écoles* (broadly on the right) and the universities and unions (broadly on the left). Whether the strike will influence either CNESER or Payan is still to be seen.

Robert Walgate

West Bank universities

Request for pledge withdrawn

The Israeli government has abandoned an earlier regulation demanding that foreign lecturers working in universities and colleges on the West Bank should sign a pledge not to assist the Palestine Liberation Organization (PLO). The announcement that the regulation would be suspended (and unofficial hints that it could be formally rescinded in the near future) came a few days after a swingeing attack by US Secretary of State George Shultz, who last Thursday called the regulation a threat to academic freedom reminiscent of the McCarthy era in the United States.

Israeli officials nevertheless deny that the events are related. Instead, they suggest the relaxation was made possible by quiet diplomacy between the civil administration of the West Bank and the university authorities. The only purpose of the regulation, they stress, had been to preserve the function of the universities as centres of higher learning and to avoid their politicization.

It is clear, however, that the Israeli government was somewhat taken aback by the strength of the international reaction to its move. One senior official reportedly expressed "surprise" at Mr Shultz's appeals to Israeli academics to join in the protests. In fact, the Israeli academic community has on several occasions come out in support of the West Bank universities — in November 1981, when the Bir-Zeit University in Ramallah was temporarily closed, a group of lecturers and research students at the Hebrew University of Jerusalem attempted, perhaps cosmetically, to organize fill-in courses for students who otherwise would have had to interrupt their studies.

The constitution of the West Bank universities and colleges under Israeli administration is complex. At the time of the Israeli take-over, there were three such establishments, Bir-Zeit (near Ramallah), El-Najah (near Nablus) and the Kadouri Agricultural College (at Jenin). These were accorded full academic status by the authorities (Bir-Zeit College became a university in 1975) and two further colleges were founded, the Islamic College in Hebron and the (Catholic) College-Frères (now the University of Bethlehem). All these are financed, indirectly, by the Israeli government, through its budget for the occupied territories, which is distributed by the civil administration of the West Bank.

Just how many lecturers refused to sign the pledge is not clear — Mr Shultz put the figure at around 120, of whom, he said 22 have already been expelled from Israel and 30 others suspended from teaching, while others were told that they would be required to leave the country when their three-month tourist visas expired. (This latter category presumably refers to new arrivals who took up their teaching posts in October this year). The Israelis, on the other hand, claim that only three lecturers were actually expelled, and that the rest were merely told that their residence permits were being withdrawn. They admit, however, that even to the newest arrivals the pledge was something that they had not foreseen when they accepted the post, and that the lecturers' refusal to sign the pledge was inspired not by a desire to assist the PLO but as a matter of principle.

"Freezing" the pledge, in the case of lecturers, however, could make difficulties with other foreign workers on the West Bank, all of whom, a government spokesman announced last week, were required to sign the pledge and had already done so in "hundreds if not thousands".

Vera Rich

International conferences

Anti-Israel moves

Rehovoot

Recent events in Lebanon threaten seriously to undermine relations between Israeli scientists and their colleagues overseas. This is one reason why Israeli scientists were relieved to hear from Weizmann Institute physicist Shalheveth Freier that despite a prolonged and often acrimonious debate on events in Lebanon at the recent Pugwash meeting in Warsaw, the organization did not adopt an anti-Israel resolution.

Instead, Pugwash adopted, after a full 20 hours of discussion in working group and plenary sessions, a far more balanced resolution than seemed possible at the outset. The resolution called for "the withdrawal of all non-Lebanese forces and the creation of a strong Lebanese government acceptable to all factions".

Freier's reception in Warsaw was thus less hostile than that of many other Israeli scientists attending international meetings in recent months. Thus one young Israeli neurobiologist arrived at a scientific conference at a French university to find a sign posted outside the cafeteria which read: "The Israelis are assassins". Many delegates echoed this view and flatly declared that they would never visit Israel again so long as the country remained "aggressive".

Strangely enough, the neurobiologist (from Tel Aviv) says he found greatest understanding from a Syrian scientist who had once lived in the city of Homs, and who was all for "smashing the PLO and the Syrian Army", presumably because several members of his family had been among the 10,000 people killed when the Assad government put down an uprising of devout Moslems in that city last year.

Some Israeli scientists have even found themselves "disinvited" from meetings during the summer and autumn. One chemist, for example, has received the following communication from a colleague in Europe: "The current situation in Lebanon is causing deep concern here. In particular, the recent actions waged by Israeli forces against the city of Beirut appear cruel and unnecessary. For that reason my group is unanimous in feeling that we cannot maintain your visit here as planned. Please understand that this is not directed against you personally."

In his reply, the Israeli chemist said that his erstwhile hosts had "the unquestionable right" to cancel the invitation, but he wondered whether they had also boycotted Iraqi scientists in the wake of their government's brutal attacks on the Kurds, Palestinian Arab scientists after the massacre of Israeli bus passengers near Tel Aviv and Israeli schoolchildren in the Galilee, or Soviet scientists because of events in Afghanistan and Poland.

Many overseas researchers seem also to have boycotted conferences held here in recent months. Thus there were only 150 participants (instead of the expected 250) at the International Symposium on Recent Developments in Perinatal and Childhood Infections and 800 participants (against 1,000 expected) at the Sixth International Congress on Hormonal Steroids. Many bodies that were thinking of Israel as a conference site for 1986–88 have also, after the furore over Lebanon, altered their plans.

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In this situation, Israeli scientists find themselves fighting two battles at the same time. On the one hand, they are trying to convince their overseas colleagues that the common judgement of Israeli policies is too harsh. On the other hand, most of them are trying to convince their fellow citizens of the need to change many of these policies.

Prominent scientists — including Professor Ephraim Urbach, president of the Israel Academy of Sciences and Humanities, and Professor Ephraim Katzir, former President of the State of Israel — played a prominent part in the successful attempt to force the appointment of a commission of judicial inquiry to probe the massacre of Palestinian refugees in Beirut. Many of these same scientists actively oppose the don't-give-back-an-inch views of the Begin government.

Even they however, are split over the question of a separate Palestinian state. Some think the emergence of such a state inevitable (or even desirable), while others would agree with what Shalheveth Freier said at the Pugwash meeting in Warsaw, where he declared: "If the general atmosphere in the Middle East continues to be uncompromisingly hostile to Israel, I suppose I should feel compelled to resist the creation of another hostile state. If, however, peace were to descend on this area, I believe that all honourable options would be open." **Nechemia Meyers**

Academic consultancy

Mass. General placates Hoechst

Washington

Massachusetts General Hospital (MGH) is going to unusual lengths to ensure that faculty consulting with outside firms does not conflict with the terms of its 10-year $50-million agreement with Hoechst AG, the German chemical company.

In the case of one faculty member just appointed to the department funded by Hoechst, MGH is demanding that his consulting with other firms be on a non-confidential basis, a condition apparently not required of other MGH faculty.

The 1981 agreement between Hoechst and MGH represents the largest joint venture so far between industry and an academic institution. Hoechst agreed to provide the money for a new department of molecular biology in exchange for an exclusive licence to any patents that result from the department's research. The agreement also requires any faculty collaboration or consultation with for-profit firms to be cleared with Hoechst.

MGH has now apparently taken it upon itself to negotiate the particulars of consulting contracts that molecular biology faculty members have with outside firms to ensure that they are acceptable to Hoechst. In the case of Dr Brian Seed, the newly-appointed faculty member, MGH has engaged the services of a prominent Boston law firm, Ropes and Gray, to negotiate changes in his consulting arrangement with Genetics Institute. Genetics Institute, the spin-off from Harvard University's short-lived plan to form its own profit-making genetics engineering company, is negotiating through its own prominent Boston law firm, Hill and Dorr.

The MGH lawyers are demanding that since Seed is free to pass on information from his Hoechst-supported work at MGH to Genetics Institute, he should similarly be free to pass on to Hoechst any information gleaned through his consultations with Genetics Institute. Seed, who says he has heard nothing about the current status of the negotiations, says the basis of MGH's position is Hoechst's concern over its ability freely to exercise its patent rights under the agreement: "Hoechst is worried that as a result of my consulting for another company I might take up in my lab some work based on proprietary information". Thus Hoechst might end up supporting some research that it could not patent.

MGH officials deny that their actions reflect any special policy towards the department of molecular biology or Hoechst. "We want to make sure that their consulting activity is consistent with the hospital's policy", says Dr Ronald Lamont-Havers, deputy general director for research. "Any consultative agreement needs to be cleared with the hospital to ensure that there is no conflict of interest."

But Lamont-Havers did acknowledge that while the hospital has advised other faculty members that their consulting contracts need to be changed, it has only actively negotiated those changes in the case of department of molecular biology faculty. And he appeared surprised to learn of the non-confidentiality requirement that MGH's own lawyers are demanding in Seed's case. "Usually, in our consulting agreements, we expect that they would receive proprietary information and would not be free to discuss proprietary information", he said.

Dr Howard Goodman, director of the department of molecular biology, denies that anyone in his department has been required to give up any consulting or that appointments have been made contingent upon modifications in an appointee's consulting contracts. And Seed seems confident that an accommodation will be reached in his case. "I doubt that my relationship with Genetics Institute will be severed", he says. But the lingering question is whether the conditions being demanded by MGH — and which Genetics Institute reportedly considers unacceptable — will make it impossible for at least some faculty consulting arrangements to continue. **Stephen Budiansky**

US defence research

Computer plan

Washington

The US Department of Defense (DoD) is adding to its existing investment in computer research several new programmes designed to counter possible Japanese domination of the field. The budget for fiscal year 1984, which is now under negotiation behind closed doors, is likely to include hundreds of millions of additional dollars for special programmes in supercomputer research and software. DoD is already supporting research in Very Large Integrated Circuits, the technology of gallium arsenide as a replacement for silicon, and other computer-related programmes.

In a speech to a professional meeting in Orlando, Florida, Robert S. Cooper, director of the Defense Advanced Projects Research Agency (DARPA) announced that he would counter the Japanese computer effort with a research programme aimed at achieving the extraordinary speed of 10,000 million floating point operations per second by 1990. Present "supercomputers" such as the Cray III and Control Data's Cyber 205 attain speeds of 100 megaflops, while the Japanese programmes aims at achieving 1,000 megaflops. DARPA's programme is rumoured to have initial costs of $150 million.

"WE WANT A WARHEAD THAT THINKS FOR ITSELF —AND GOES FOR TOKYO!"
DEPARTMENT OF DEFENSE

DARPA supports basic research and exploratory development for the directorate of Defense Research and Engineering and would not, therefore, be involved in applications. Possible defence applications include warhead delivery systems capable of "deciding" for themselves which targets to attack, and thus not dependent on vulnerable telemetry systems.

There is also to be a substantial new programme in the development of software to be managed by a new office to be established within the Defense Research and Engineering directorate. According to a report from the office of Dr Edith Martin, the deputy under-secretary at the Pentagon with responsibility for research and engineering, it is planned to spend an extra $30 million on software development in the financial year beginning on 1 October 1983, to provide further funds in succeeding years and to establish a military software institute. It is estimated that between $5,000 million and $6,000 million worth of software is already "embedded" in defence systems, and that the amount will rise to $32,000 million by 1990.

Increased funds are also likely to be

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made available for the Very High Speed Integrated Circuits programme as well as for the research and development programme based on gallium arsenide, a material that offers faster semiconductor devices and may be more resistant to disturbances such as electromagnetic pulses.

The Pentagon's efforts to match the Japanese effort in advanced computers is mirrored by two initiatives by the computer industry. However, the plan of 15 computer companies under the leadership of the Control Data Corporation to invest between $50 and $100 million a year in software and hardware development has not yet become a reality even though the founders are confident that the US Department of Justice will decide that the proposed consortium, the Microelectronics and Computer Technology Corporation, does not violate anti-trust laws.

The non-profit Semiconductor Research Corporation, set up by 13 computer companies earlier this year, has however begun making grants to academic institutions considered to be centres of excellence in computer development. Last week, the corporation announced grants for developments in large-scale integrated circuit technology to Cornell University, the University of California at Berkeley and the Carnegie-Mellon University.

Deborah Shapley

French win contract

The British Central Electricity Generating Board (CEGB) has commissioned the design and safety study of the pressure vessel for the planned Sizewell B pressurized water reactor (PWR), Britain's first, from Framatome, the main constructor of the 40 or so French PWRs.

Thus Dr Walter Marshall, now director-general of CEGB, has proved true to his word. In March, when he was chairman of the United Kingdom Atomic Energy Authority, and head of the PWR task force that tidied up the Sizewell safety design, he was questioned about the problems that Framatome had had with cracks under the pressure vessel cladding. This would be just the time to buy from the French, said Marshall — because "they have been caught with their pants down". The problem was one of heat treatment, and has now been solved, Marshall believed, probably leaving Framatome with a better understanding of such vessels than other manufacturers.

From his position as head of CEGB, Marshall has now approved the offer of the design contract, worth £213,000, to Framatome. This will not be complete until May 1983, after the January 1983 Sizewell inquiry, but — according to Framatome sources — a report amounting to "the bulk" of the study will be ready in time for the inquiry.

Robert Walgate

MRC annual report

Universities stand to gain

Like publishers, heads of research councils are usually a gloomy bunch when it comes to public statements on the financial position of their organizations. But at last week's launch of the annual report of the UK Medical Research Council (MRC), the council's secretary, Sir James Gowans, was remarkably cheerful about MRC finances and even offered a helping hand to the universities.

The hand-out, which all told could amount to 1 per cent of the annual budget of MRC, is offered to university research teams, in particular those already within the system of dual support by MRC and university, whose cutting edge has been dulled by diminished support from the university. To restore their competitiveness until such time as university finances are again healthy, MRC is offering to convert such teams into MRC Groups, providing them with up to £50,000 per annum.

The scheme, advertised earlier this year, has attracted some twenty applicants, almost all of them already substantially financed by the MRC. To ensure that the money is awarded to cases of genuine hardship, the vice-chancellor of the university has to make the application and to promise that he is not trying to free resources which will be redeployed into unrelated activities. Although no decisions have yet been made it is likely that the support awarded to MRC groups will cover such items as radiochemicals, an electron microscopist, an animal-house technician or even a departmental secretary.

MRC seems willing to spend up to £1 million a year in this way. Where will the money come from? Nowhere in particular, according to Sir James Gowans. Nevertheless, two main sources seem likely.

The first would be the £1 million that would be saved were MRC to withdraw its support of the European Molecular Biology Laboratory (EMBL) in Heidelberg. The suspicion that it might do so arises from an instruction from the Advisory Board for the Research Councils telling MRC to review its participation in EMBL. Asked why he thought the review was called for, Sir James Gowans would say only that the question should be addressed to the Advisory Board — of which he is a member.

Alternatively, it might be surmised, MRC could support its proposed groups by spending less on project and programme grants to university teams. According to the annual report, "the growing volume of applications for support . . . suggests . . . that some good work will continue to go unfunded" but in fact no really good project ever seems to fail to be supported.

Looking ahead, Sir James Gowans drew attention to the review next year of the five-year agreement between MRC and the biotechnology company Celltech. The agreement runs until 1985 and Sir James thinks "it is going all right". He also mentioned the National Institute of Medical Research (NIMR) which this year will consume about £9 million of the £106 million MRC budget. Sir James expects Dr D.A. Rees, formerly principal scientist at Unilever's Colworth laboratory but director of NIMR since 1 October, to increase the links between NIMR and industry and to open facilities at NIMR to university researchers. In addition, the structure of NIMR will be reorganized during 1983.

Peter Newmark

Rostow a "dove"

Washington

Part of the reason for the apparent stalemate of the Geneva arms control negotiations seems to be a dispute between a group of conservative senators and Mr Eugene V. Rostow, director of the Arms Control and Disarmament Agency (ACDA). The dispute may come to a head as soon as 29 November when a group in the Senate led by Senator Jesse Helms (Republican, North Carolina) will try to send Rostow a warning that he is too doveish towards the Soviet Union — and put the President on notice as well.

The Helms group first signalled its discontent in March, when the Senate Foreign Relations Committee considered the nomination of two long-term ACDA officials kept on by the Administration at Rostow's urging. Helms complained that Robert Grey, nominated as deputy director, and Norman Terrill, nominated as assistant director for nuclear weapons policy, were hold-overs from the Carter Administration and too doveish.

Since then, confirmation of the two nominees has been held up in the Senate because of Helms's objections. When Congress reconvenes on 29 November for about a month, there will be a move to bring the nominations to the floor of the Senate, which is possible only if Helms withdraws his objection. There is a rumour that only the Grey nomination will be called up. The Helms faction, which would prefer General Edward Rowney to have Rostow's job, seems to have calculated that postponing Terrill's confirmation as acting assistant director will continue to embarrass Rostow and, in particular, prevent him from taking a decisive position on arms control.

If the two nominations are not confirmed by the time Congress finally disbands, the whole nomination process will have to begin all over again in the new Congress next February.

Deborah Shapley

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Soviet psychiatric abuse

Allegations met by red tape

The Soviet All-Union Society of Psychiatrists (AUSP) has adopted a new approach to accusations by human rights groups about the confinement and "treatment" of dissidents in Soviet mental hospitals. Instead of rejecting all such allegations out of hand, it has supplied the World Psychiatric Association (WPA) with details of two alleged cases and has promised information on four more.

The fact that the data appear incomplete, and the extreme slowness with which they have been made available, have evoked considerable suspicion among some members of WPA. Is this a genuine change of heart or simply a delaying tactic on the part of the Soviet Union to forestall moves by several member bodies — including the Royal Collge of Psychiatrists and the American Psychiatric Association — to have AUSP expelled or suspended from WPA?

This is not the first time that AUSP has professed itself willing to cooperate with WPA over allegations of psychiatric abuse. During the last world congress, in Honolulu in 1977, the Soviet delegate to the general assembly handed round a substantial document containing "confidential" clinical data on twelve dissidents whose cases had frequently been cited as "alleged victims of psychiatric abuse", in the hope of convincing the delegates that the dissidents were in fact suffering from severe psychoses. The Honolulu congress narrowly passed a motion of censure against the Soviet Union, and set up a special review committee to monitor allegations of political use of psychiatry.

AUSP, however, has never acknowledged the competence of this review committee, and the executive committee has had to assume much of the responsibility for investigating allegations involving the Soviet Union. The work of the review committee so far was presented at an executive council meeting in Marrakesh earlier this month in a special report by the secretary general, Professor Peter Exner, which was reportedly condemned by the Soviet observer Professor Marat Vartanyan as being "too negative". For those member societies that have submitted allegations of psychiatric abuse to WPA, follow-up of the original complaint is hindered by what the complainants see as an unreasonable insistence on professional confidentiality.

Whether this strict insistence on professional ethics is a result of Soviet pressure is not clear. Ironically, in many cases, the complaints have been originated by the victims themselves asking to have the diagnoses made public, in order to reveal their dubious medical basis. There are growing fears among member societies of WPA that the executive council's over-scrupulous stance on confidentiality may unwittingly be going against the interests of other individuals whose cases are under review.

Vera Rich

Romanian emigrants

Cash demands

A decree promulgated earlier this month by the Romanian Council of Ministers seems certain to hamper the movement abroad of Romanian scientists. The decree requires that Romanian emigrants should repay (in hard currency) the costs of their secondary and higher education.

The decree is reminiscent of that in the Soviet Union some ten years ago designed to recover higher education fees from Jews wishing to emigrate to Israel. The Soviet ruling evoked widespread protests abroad, including the imposition of credit restrictions by the United States, and was allowed to lapse less than a year later.

The Romanian decree is likely to attract even more vehement criticism. Already the US State Department has called it a "draconian measure", contrary to the letter and spirit of the Helsinki accords, and has hinted that it could hamper the renewal, next year, of Romania's recently acquired "most favoured nation" status.

The condition that higher education costs, however calculated, should be repaid in hard currency, will be virtually impossible to satisfy. (It is illegal for Romanian citizens to have hard currency in their possession.) The most probable objective of the decree is to clamp down on emigration, a conclusion borne out by the reports from Bonn that the Romanian government has given notice to end the agreement under which up to 10,000 Romanians of German origin are allowed to emigrate each year.

The effects of the new decree, moreover, are liable to be felt in all countries that have scientific exchange programmes with Romania. The decree, for example, lays down that a scholar allowed to travel to an international conference who fails to return on schedule will be treated as an illegal emigrant, and will be sued for the cost of his or her education both at home (presumably with the confiscation of assets) and in the new country of residence. The relevant clause of the decree is phrased ambiguously, and could be construed to apply retroactively to those who have left the country in recent years, especially since the mid-1970s.

Vera Rich

British Astronomer Royal

Professor F. Graham Smith, now the director of the Nuffield radio-astronomy observatory of the University of Manchester at Jodrell Bank and until earlier this year director of the Royal Greenwich Observatory, was this week appointed Astronomer Royal of England. He succeeds Sir Martin Ryle, who resigned the post as from October. One of the ironies of the new appointment is that Sir Martin's appointment as Astronomer Royal was intended to mark a separation of that post from the directorship of the Greenwich Observatory.

EEC research council

Cash injections planned

Brussels

Despite the row over the budget for Super-Sara, the project for investigating what happens when light-water reactors lose coolant (see *Nature* 11 November, p.99), the November meeting of the EEC science ministers approved a near-record number of research programmes. With one or two minor reservations the pilot stage of the Esprit programme on long-range research on information technologies (see *Nature* 3 June, p.352), the 4-year indirect action programme in favour of developing countries, a programme for the development of a translation machine and finally the experimental phase of the project to stimulate the European Community's scientific and technical potential were all given the green light.

The programme to stimulate science and technology is to last 2 years and will have a budget of 7 million European Currency Units (£3.7 million), an increase of 40 per cent over the amount originally demanded. Before the programme can be implementated, however, the European Parliament must be given time to express an opinion and a formal proposal must be made by the Commission.

The money will be used to test out the Commission's plans to operate a new fund which it can invest rapidly in any projects considered promising without having to resort to the usual cumbersome system of elaborating formal programmes. The experimental phase will test out this concept in seven areas which are considered important and in need of "stimulation" — pharmacobiology, solid state physics, optics, combustion, photometry, photoacoustics, interface phenomena and climatology.

Under the new system, a consultative committee (CODEST) made up of outstanding authorities in science and technology will advise the Commission on how to spend the money. Applications for research grants, subsidies for laboratory twinning, development contracts, subsidies for research teams, seminars and workshops will all be considered.

If the experimental phase is successful, the scheme will be extended for the years 1984–87 and a figure — perhaps as much as £12 million, or 5 per cent of the Community's joint annual research expenditure, will be set aside each year.

The scheme was first agreed in principle at the last Council on 30 June, as was the Esprit programme, for which a pilot stage was agreed by the November Council, with 11.5 million ECU (£6 million) available from the EEC budget. Universities and companies will be expected to contribute at least 50 per cent of the cost if they seek support in carrying out research. Money has been set aside for pilot projects in advanced electronics (two projects), software technology (three), advanced information processing (three), office automation (four), computer integrated manufacturing (three), and information exchange systems. Each contract must contain at least one industrial participant.

A 5-year programme budgeted at 16 million ECU (£8.5 million) will now also start, aimed at building an advanced translation machine to "extend the frontiers of computational linguistics". The advantages of such a machine are evident when one considers that about a third of the 16,000 EEC civil servants are currently involved in translating or interpreting the seven Community languages.

And for the first time, the EEC will have a joint research programme in aid of developing countries (see *Nature* 15 July, p.218); 40 million ECU (£21 million) will be spent on two sub-programmes between 1982 and 1986: tropical agriculture and medicine, health and nutrition in the tropics. The programme has raised doubts in the minds of some delegations and at the European Parliament, since despite its title the programme will mostly benefit laboratories in the EEC rather than directly helping developing countries. However, the agreement to have the programme at all is an important step towards realizing the ambition of Viscount Etienne Davignon, European Commissioner with responsibility for research and development, to make work dedicated to assisting the developing world one of the seven pillars of the EEC's research and development strategy for the 1980s. **Jasper Becker**

French research and development

Can the good times last?

France's foreign debt is now estimated to be FF 300,000 million (£25,000 million), double the figure of a year ago, and the balance of trade deficit was running at FF 12,000 million (£1,000 million) a month in September. But still France continues to promise to spend, spend, spend on research and development. Earlier this month the National Assembly voted the government civil research budget for 1983: it will be FF 52,200 million (£4,300 million), 28 per cent up in money terms over 1982, and 17.8 per cent up in real terms. This is not a jot different from that previewed in the July "law for research" but the economic problems are raising fears that the figures will prove, in the end, a mere façade.

For instance, this year the finance minister ordered a freeze on government spending which — so far as science was concerned — held back a quarter of the planned budget for new research equipment. Caught unawares, laboratories which had already made orders found themselves falling rapidly into the red — until the minister of research and industry was able to claw back the 25 per cent just a few weeks ago. Other ministries, such a the ministry of national education, wer not so successful. There is talk next year o a freeze of 30 per cent. Will the researc minister claw that back too? Some dout it, despite the fact that in France, researc and technology appear to have become th new faith.

Nevertheless the budget figures ar worth analysing as indicators of intentio Jean-Pierre Chevènement, the researc and industry minister, plans to cut his cak of FF 52,200 million (£4,300 million) fo 1983 into FF 32.5 million for civil researc and development, and FF 19.7 million i support of his industrial and energy polic

The research budget proper — mone for the research councils — will be up 1 per cent to FF 23,500 million. The figur includes many salaries. There will also b fiscal support to companies undertakin more research (in particular a rather back handed relief from a new "tax o fortunes"). This and other measures ar intended to help industry boost its ow research budget by 8 per cent next year, a envisaged in Chevènement's grand plan.

Basic science is not the principal ben ficiary of the increases, although it is no neglected. The French government fundamental objective is the renewal o industry through long-sighte government-managed investment. So th greatest increase — 62 per cent — with the FF 32.5 million goes to Chevènement seven "*programmes mobilisateurs*" which cover new energies, electronics, bi technology, research and innovation f the Third World, research on the co ditions of labour, the promotion of th French language in science and technolog and general support for industri innovation. These are the workhorses Chevènement's policy, although nobo seems sure exactly how the cash will harnessed to research. **Robert Walga**

French civil research and development budget (thousand million FF)

	Budget 1982	1983	Increase (per cent)
"*Programmes mobilisateurs*"	5.3	8.6	62
Applied research	3.7	4.5	22
Technology programmes (nuclear, aerospace and oceans)	6.6	8.1	23
Other	5.1	5.5	8
Total government civil research and development	25.4	32.5	28

FF 11.75 = £1. Amounts are in current francs; inflation cuts increases by about 10 per cent.

Direct support for French scientists and the research organizations (thousand million FF)

	Budget 1982	1983	Increase (per cent)
Direct ministerial support	1.16	1.49	28
Centre National de la Recherche Scientifique	5.95	6.94	17
Commissariat à l'Energie Atomique	5.26	5.85	11
CNES (space)	2.15	2.74	27
INRA (agriculture)	1.51	1.74	15
INSERM (medical)	1.04	1.25	21
ANVAR (transfer of research to industry)	0.83	0.99	19
ORSTOM + GERDAT (Third World)	0.76	0.89	17
ADI + INRIA (informatics)	0.44	0.48	9
CNEXO (oceans)	0.40	0.49	22
AFME (new energies)	0.30	0.39	30
Institut Pasteur (Paris)	0.14	0.17	21
Other	0.11	0.12	10
Total	**20.05**	**23.54**	**17**

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In vitro fertilization

MRC tests the climate

A set of guidelines that gives qualified approval for researchers to perform experiments on human embryos obtained through *in vitro* fertilization was issued last week in a statement by the Medical Research Council (MRC). The guidelines are likely to please scientists working in the area, but the British Medical Association (BMA) has expressed concern that they may be too liberal for its members.

The MRC statement, published in the *British Medical Journal,* states that clearly defined research on embryos which are not intended for return to the uterus is ethically acceptable if it is "directly relevant" to clinical problems. It also allows the deliberate fertilizing of human ova *in vitro* for specified and approved research projects, as well as the use of fertilized ova left over from therapeutic programmes, provided that informed consent is obtained from both parents.

The guidelines were drawn up by an MRC advisory group under Professor Geoffrey Dawes. They are intended to apply only to MRC-supported scientists, who must in any case obtain specific approval for any proposed experiment from local scientific and ethical committees. Nevertheless, MRC's ideas are likely to be influential as a basis for discussion within the numerous other committees set up to consider the implications of recent developments in the field of human fertilization, such as the British government's Warnock committee (see *Nature* 29 July, p.408).

The MRC proposals allow *in vitro* culture of human embryos only up to the implantation stage, a clearly defined phase of development corresponding roughly to the time at which embryonic tissues become distinct from those of the placenta and organs start to form. This stage is reached at 13–14 days after fertilization, considerably older than the 9-day-old embryos that were at the centre of a recent storm after Dr Robert Edwards announced that he had kept and observed "spare" embryos initially produced for reimplantation. The new guidelines also permit experiments on interspecies formation — valuable for studying defective sperm — but in this case it is recommended that embryos should not be allowed to develop beyond the early cleavage stage. Experiments on techniques for freezing embryos for storage will be allowed provided that embryos are not stored for future unspecified research.

Dr Anne McLaren, director of MRC's Mammalian Development Unit at University College London, and a member of the advisory group that drew up the guidelines, has pointed out that research aimed at increasing the safety and the success rate of "test-tube baby" programmes is concerned with the embryo before implantation; workers in this area should not therefore find the guidelines overly restrictive.

The BMA, while accepting the MRC guidelines as "a valid contribution to the debate", appears to have reservations about how acceptable they will be to its members: a spokeswoman expressed the view that the MRC guidelines "will do nothing to allay public fears".

The BMA has its own committee under the chairmanship of Professor Peter Quilliam, seeking to establish guidelines for the medical profession, and is unwilling to make specific comments until the committee has made its report; however, it seems possible that the adoption of the implantation stage as what is in effect a criterion for the start of a human life could be a point of disagreement. In the meantime, BMA members are not banned from involvement in MRC projects. There is clearly a lot of arguing still to be done: in the foreseeable future there are going to be strong reasons for wanting to experiment on embryos considerably beyond the implantation stage. **Tim Beardsley**

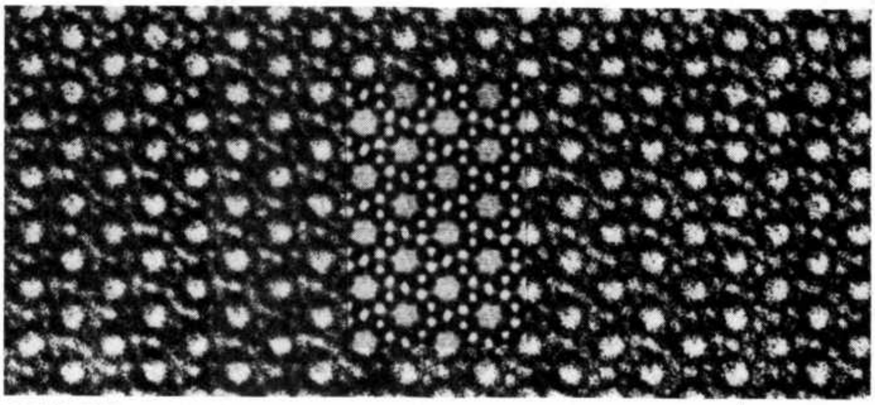
Research article triggers dispute on zeolite

Washington

An experimental result published in the 8 April 1982 issue of *Nature* has set off a legal battle between Union Carbide and Mobil Oil that may involve hundreds of millions of dollars in patent royalties.

The dispute is over synthetic minerals known as zeolites, which have become increasingly valuable as catalysts in the production of chemicals and liquid fuel from coal. The zeolites, which are crystalline aluminosilicates, have large regular pore structures making them highly selective in binding molecules of a desired size.

In 1977, Union Carbide obtained a patent for what it claimed was a novel form of zeolite called "silicaltite". But according to the analysis reported in the *Nature* article (C.A. Fyfe, J.M. Thomas *et al., Nature* **296**, 530), silicalite is in fact "identical" to a family of zeolites patented by Mobil in 1972. Mobil's zeolites, known as ZSM-5, are vital to Mobil's methanol-to-gasoline process.



Mobil's ZSM-5 zeolite. The diameter of the largest pores, lined with active sites, is 5.5 Å. A computer-simulated image is inset. (Photo: G.R. Millward/J.M. Thomas, University of Cambridge.)

Mobil then informed Union Carbide that it considered silicalite an infringement on its patent; Union Carbide responded on 21 September by suing Mobil in the Federal District Court in New York City, asking for a declaratory judgement that silicalite does not infringe on Mobil's patent. In a counterclaim filed 2 weeks ago, Mobil officially entered its charges of infringement.

Neither side is willing to discuss the case in any detail; Mobil has refused even to provide a copy of its response and counterclaim, which are part of the public record. But an industry expert on zeolites gave *Nature* the following explanation of the facts surrounding the case. Mobil's ZSM-5 patent covers zeolites with silicon-to-aluminium ratios from 10 to 4,000. Union Carbide claims that silicalite is 100 per cent silica within the zeolite framework; any aluminium that happens to be present in silicalite, Union Carbide says, is outside the framework, and thus does not play a part in silicalite's essential properties.

What Fyfe *et al.* showed, however, is that aluminium is present in silicalite in least two distinct tetrahedral environments — which means that aluminium *is* in the framework. Aluminium outside the framework is octahedral. "The silicalite structure is shown to be essentially indistinguishable from that of the siliceous zeolite catalyst ZSM-5", Fyfe *et al.* concluded. Their work involved the use of ^{29}Si and ^{27}Al NMR spectroscopy.

The speculation is that Union Carbide made the preemptive strike of suing Mobil after receiving an order for silicalite. The threat of a patent infringement suit would have to be removed from Union Carbide and the purchaser before a sale could go through. Union Carbide refuses to say whether an order has been received or whether any silicalite has been sold.

Stephen Budiansky

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CORRESPONDENCE

This space was occupied by a letter withdrawn after the press deadline (see page 302)

South African ban

SIR — I do not wish to enter the debate about British participation in astronomy in South Africa but I must object to some statements in the letter from Dr David Evans (*Nature* 11 November, p.102).

He states that boycotts of the type proposed are usually ineffective. The only boycott properly applied to South Africa has been in the field of sport, and this has been most effective, leading to integration in most branches. Many of us believe that the same kind of pressure applied to academic circles would achieve a similar result with beneficial consequences for the education of non-whites in that country. An academic boycott might be a blow to some sections of the community (almost all white) but would be welcomed by many others (almost all black) who derive great moral encouragement from manifestations of external sympathy with their cause.

He is of course right when he says the South African government has a problem of "terrorist activity", although this is a term one prefers not to apply to people who are simply trying to achieve equal rights for themselves. It must of course be evident that South Africa has brought this activity on itself; the problem would not have existed had it not chosen to suppress the majority of its people by refusing to provide ordinary democratic rights. Dr Evans is most certainly a victim of South African propaganda when he refers to the activists as being "inspired by the Soviet Union". Has he learned nothing at all from events in Zimbabwe? African people want independence and self-determination, not replacement of one dominant force by another.

Finally, he may believe that the best hope for a peaceable solution lies in an increase of cultural links from abroad. He must know that South Africa used to enjoy these links fully as part of the British Commonwealth and a respected member of the international community. It chose to isolate itself by inflicting a policy of apartheid on most of its inhabitants. Why should increased contact with the rest of the world achieve more now than it did 35 years ago? R. HOFFENBERG

University of Birmingham,
Birmingham, UK

Naming enkephalins

SIR — It is easy, as Morley (*Neuropeptides* **1**, 231-235; 1981) has pointed out, to be misleading in attempting to specify the structures of enkephalins and their analogues. The commonest confusion may be caused if [Met]enkephalin, for example, is written as Met-enkephalin. This is because IUPAC-IUB recommendations, used throughout peptide chemistry, specify square brackets for substitutions and forms such as "Met-" for N-terminal extensions. Thus the form "Met-enkephalin" might well be necessary for extension of the basic sequence Tyr-Gly-Gly-Phe-Xaa to Met-Tyr-Gly-Gly-Phe-Xaa. The form [Met]enkephalin seems convenient as a simplification of the strictly systematic [Met^5]enkephalin, which should be used in contexts where ambiguity is conceivable.

The recommendations for C-terminal extension and for partial sequences are illustrated by the identity of [Leu^5]enkephalinyl-Arg-Arg with dynorphin(1-7) peptide.

References to the many printings of the 1966 IUPAC-IUB recommendations are given by Morley. They are now under revision, but there is no proposal to change appreciably any of the methods of designating N- and C-terminal extension of peptides, substitutions, deletions and partial sequences. The new recommendations are likely to be published in 1983. H.B.F. DIXON

Nomenclature Committee of IUB,
Cambridge, UK

Chinese seismometry revisited

SIR — I write with reference to an article on a Chinese seismometer featured in "*Nature* 100 years ago" (ref.1). You may be interested to learn that the original article carried some errors. According to Joseph Needham[1], "Choko" was the Japanese version of the name of Chang Hêng (AD 78-139), a noted astronomer, and his seismometer dates from AD 132 rather than AD 136. Several reconstructive drawings are illustrated in Needham's book and in 1910 another illustration was presented to this museum by the Japanese Imperial Department of Education.

We currently exhibit a sectioned reconstruction of the seismometer (see below), given by the BBC, which has been demonstrated on television. In 1980 the Chinese Seismological Association presented a small statue of Chang Hêng to the Science Museum; this too is on exhibition. The seismometer is also figured on one of a set of Chinese postage stamps, on display alongside Chang Hêng and his seismometer in our Geophysics Gallery.

ANITA MCCONNELL
(*Curator*)

Science Museum,
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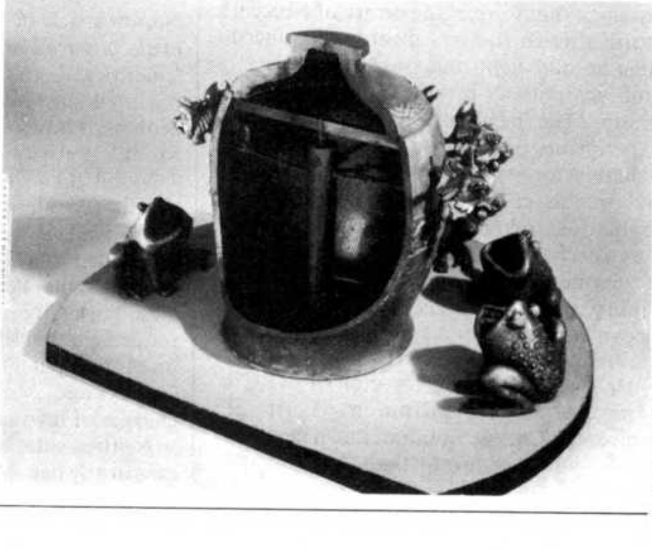

Science Museum

What future for the chaos concept?

Is classical chaos a kind of premonition of quantum uncertainty or something separately? Now it may be possible to tell by means of an experiment, not endless argument.

What is chaos? During the past few years, physicists (not to mention philosophers of science) have been much preoccupied by the recognition — "discovery" would be the wrong word — that there are many macroscopic systems whose future behaviour cannot be accurately predicted. The old saw is that fourteen-day weather forecasting is impractical because, over such a time-scale, disturbances initially as negligible as the flapping of a single seagull's wings may turn out to have a worldwide effect. In meteorology, the problem is to know which seagull's flapping wings matter. There are two opposing answers to this absorbing question. Some will hold that the difficulty of calculating the effect of a pair of flapping seagull's wings is temporary; better mathematics, or larger computers, will make it possible to tell whether a seagull's wings have a greater effect when the Sun is at its zenith than at night, or whether mid-latitudes are more sensitive to disturbance than either tropical or polar latitudes. Others, however, take a different view. There are so many seagulls, so randomly distributed over the surface of the Earth but so constantly flapping their wings, that even if the mathematics of the meteorological problem were exactly soluble, the initial conditions would be intrinsically unknowable and the time-course of the development of the macroscopic system therefore intrinsically indeterminate. In this strictly classical sense, physical chaos is a way of referring to circumstances in which applied mathematics is imperfect — either the differential equations cannot be solved with sufficient accuracy or they (or the boundary conditions that should apply) cannot be accurately defined.

Inevitably, such circumstances are reminiscent of what happens in quantum mechanics: indeterminacy abounds. But to suppose that the similarity is meaningful would be mistaken. Quantum chaos is, after all, well-defined. You can measure the position of a particle but then not its momentum, or its momentum but then not its position, or some mixture of the two with uncertainties determined (apart from numerical constants) only by the mass of the object and Planck's constant. Chaotic macroscopic physics is distinct from that line of experiment (or *Gedanken*-experiment) but not unconnected with the reason why the generalizations of thermodynamics were fashionable in the nineteenth century, before people pretended to be able to solve complicated differential equations exactly. That, surely, is how sober physicists would argue.

But if quantal and classical chaos are independent phenomena, should it not be possible to find evidence of some kind of analogue of classical chaos in the behaviour of quantum systems? This is the question taken up by R.V. Jensen of Yale University in one of this month's issues of *Physical Review Letters* (**49**, 1365). Such problems are potentially important, as can easily be told by asking what will happen in the course of time to a large polyatomic molecule whose total vibrational energy is, say, either just above or just below the energy necessary to break one of the covalent bonds that hold the vibrating structure together. If the threshold is not quite surpassed, on classical physics the molecule will never shake itself apart. In a quantum framework, however, there will be a finite chance that the molecule will tunnel through from an entirely cohesive state to one in which there are two separate pieces. If the total vibrational energy is just greater than the threshold, quantum mechanics has nothing fresh to say — the rate of transition from one state to the other is greater, but the chance of sliding over a shallow barrier is not very different from that of tunnelling through it. On the face of things, only the case of a classical polyatomic molecule whose energy is just above the binding energy of its weakest bond is interesting; then, on the face of things, it can only be a matter of time, perhaps a long time, before the molecule falls apart. The argument about the classical problem of chaos is essentially that of whether the equations of motion or the initial conditions can ever be sufficiently well-defined as to make the calculations of a single molecule's fate determined. Most people would settle for an ensemble of molecules and some form of thermodynamics, statistical or otherwise.

Jensen's question is different — that of whether a quantum system can be expected to behave with a degree of unpredictability transcending what Heisenberg uncertainty would require. In principle, such questions could be resolved by experiment. How frequently does a polyatomic molecule whose Hamiltonian is well-defined choose to shake itself apart? How easily can an electron be detached from a polyelectronic atom? Unfortunately, such systems, in which the quantum numbers are necessarily large, are complicated by the inherent quantal uncertainty. What Jensen has done is to devise a hypothetical experiment in which it should be possible to search for quantum chaos without the interference of quantum uncertainty in Heisenberg's sense. Moreover, Jensen even promises that his experiment will be carried out.

The system considered is unusual, to say the least, and consists of the free electrons that must be supposed to exist above the surface of liquid helium. Such an electron is attracted to the surface by its electrostatic image charge, but prevented from penetrating the surface by the Pauli exclusion principle. Accordingly, in classical language, the electron oscillates between the surface and a distance (say a nanometre) determined by its kinetic energy. In quantum language, however, the electron is bound loosely in a potential well and, like an electron in any other kind of box, has a series of stationary states whose energy levels can be calculated by the manipulation of Schrodinger's equation. (Technically, the problem is not as simple as it sounds, for the potential energy is inversely proportional to the distance from the surface, as with any other electrostatic charge attracted by its image.) Jensen solves the problem by transformation of the dynamical coordinates to action-angle variables; those with an algebraic interest will discover that the Hamiltonian (total energy expressed as momentum position) can validly be written in terms of a pair of other variables one of which is the square root of the maximum amplitude of the electron's excursion from the surface, in which case the corresponding Hamiltonian variable is an angle.

The upshot is intriguing. Jensen supposes that electrons bound in this way near the surface of liquid helium are also perturbed by microwave radiation (whose wavelength is necessarily greater than the maximum excursion of the electrons and whose spatial variation can therefore be neglected). In classical language, electrons bound like this should be shaken free by the processes that lead to classical chaos. The interesting question is whether classical chaos persists in the quantum regime or whether it merges with it. The recipe that follows from Jensen's work is to point a beam of microwave radiation at a liquid helium surface and to observe the rate at which electrons are shaken loose, or microwave power absorbed. The phenomenon should be evident at about 320 GHz if the measured dielectric properties of liquid helium are applicable. Rarely can a point in the philosophy of physics have been reduced to such a simple measurement.

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Mantle methane — fool's gold?

from the Planetary Sciences Unit, University of Cambridge

THE controversial idea perpetrated by Professor Tommy Gold of Cornell University that hydrocarbons have their origin in the outgassing of methane from the Earth were the subject of a recent discussion meeting* at the Geological Society. Gold believes that the Earth contains in its interior a vast reservoir of primitive methane (CH_4) incorporated at the time when the solid body first formed. He suggests that over geological time, higher-molecular-weight hydrocarbons might be formed from a gradual leak of this primordial gas — non-biological petroleum appears from gas on its way out of the Earth's interior rather than being formed from increasing maturation of biological debris on the way down from the surface.

Gold's ideas have provoked a lively debate in the scientific community (speakers against included G. Eglinton, University of Bristol; J. Watson, Imperial College, London; and M. Schoell, Hannover) and, not surprisingly, have generated interest within the oil and gas industries. Although a general air of scepticism among both academics and commercial companies prevails, convincing evidence to negate the idea completely does not seem to be forthcoming. Since it is Gold's habit to dismiss arguments against as depicting special circumstances, the often heated exchanges continue. The Natural Environment Research Council has opted to maintain an open mind and a committee has been formed to consider deep-source gas under the chairmanship of Sir Peter Kent, who, in his policy statement to the meeting, pointed out that the wide range of geological circumstances in the UK provides a very suitable platform for appropriate investigations.

Gold proposes that methane, which is stable at high confining pressures and low oxygen fugacity, rises to the surface, not in a continual stream, but through a series of isolated traps. When the pressure in a particular enclosure exceeds a certain value, some of the gas is discharged into the next higher level by a momentary opening of the interconnecting pore spaces. Depending on the pressure–temperature–oxygen fugacity relationships experienced by the ascending gas, it will either reach the surface as methane (which may even spontaneously ignite if large volumes escape into the atmosphere) or carbon dioxide.

Gold's theory gains support from several independent lines of evidence. First, methane has been found outgassing from mid-ocean ridge zones, crustal rifts, volcanoes and other areas of tectonic activity and also from a Russian deep drill-hole. The presence of methane at certain mid-ocean ridges correlates with that of ^{3}He (J. Welham and H. Craig *Geophys. Res. Lett.* **6**, 829; 1979) which is universally accepted as a primordial telluric gas component incorporated at the time of the Earth's formation. The inference therefore is that methane is primordial in origin, exists deep within the mantle and perhaps even acts as the 'carrier' of the ^{3}He.

Second, he argues that various He–CH_4–N_2 relationships across some oil and gas fields cannot be explained unless two pre-existing, two-component reservoirs are mixed together in a deep location.

Third, at certain localities, Gold envisages that methane outgassing through the crust, is converted to CO_2 by methylotrophic bacteria and is then incorporated into carbonate cements. These deposits have unusually light carbon isotopic composition (-35 to -25°/oo), thereby making them distinct from normal carbonate sediments. The methane from which they are produced shows a depth-dependent increase in δ^{13}C (from -80 to -40°/oo) which can be explained by the continual removal of relatively heavy carbon to form carbonate as the methane rises to the surface. (All isotope measurements herein are relative and defined according to the δ convention, where

$$\delta^{13}C\ (^\circ/_{oo}) = \left[\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{std}} - 1\right] \times 1000).$$

Thus, a single methane source is invoked to interpret the data. Opponents of the idea allude to the possibility of two methane origins to explain the trend — a deep thermogenically produced gas from a sedimentary source and a near-surface methane resulting from bacterial processes. Gold maintains, however, that the quantities of methane needed to produce some of the known carbonate deposits (for example, Cement, Oklahoma) would be vast (enough to fuel America at its current rate of consumption for 1,000 years) and therefore cannot have been generated by bacteria or cracking of higher hydrocarbons. If one accepts the Gold hypothesis that primordial methane alone is responsible for carbonate generation, then it is extremely interesting to note that in all cases the deposits lie directly above oil reserves. (Gold considers this as 'smoking gun' evidence that non-biogenic methane contributes to hydrocarbon formation.) However, even though only five per cent of all organic material produced in the geological environment (G. Eglinton) can be proved by specificity of carbon skeleton to have a biological origin, the idea that the remaining material has been produced non-biogenically has still not found favour.

It is by no means universally accepted that the carbonaceous gases escaping from the Earth's surface are primordial in origin. The alternative view is that they are from recycled material — unfortunately the residence time for carbon in this recyling event is estimated to be greater than 10^8 years (M. Javoy *et al. Abstr. 5th int. Conf. on Geochronology, Cosmochronology and Isotope Geology,* p.173, Japan, 27 June–2 July 1982) and so no useful information would be yielded by ^{14}C measurements. However, the stable isotope composition of methane (both D/H and ^{13}C/^{12}C determinations) may help to resolve the issue. Schoell certainly argued convincingly that the isotope systematics of methane in Lake Kivu cited by Gold as correlating with ^{3}He could be accounted for by reduction of magmatic carbon dioxide together with gas derived by fermentation.

It may be ironic that Gold believes that some of his strongest evidence for a primordial origin (as above) derives from stable isotope data, given the paucity of relevant information. Traditionally, the primordial δ^{13}C value of the Earth is quoted as being between -5 and -7°/oo. However, diamonds, which are undoubtedly of deep-seated origin and might be expected to have once resided close to the primordial carbon reservoir, display a range in δ^{13}C values from 0 to -30°/oo (D. Deines *Geochim. cosmochim. Acta* **44**, 943; 1980) for reasons as yet unknown. Furthermore, the δ^{13}C value of ^{3}He-correlated methane outgassing from mid-ocean ridges is found to be about -18°/oo (J. Welham *EOS* **61**, 996; 1980). How these data relate to δ^{13}C values from the other major carbon reservoirs [-7, -27, -41°/oo for atmospheric CO_2, CO and CH_4, respectively, or about 0°/oo for most carbonate deposits, around -25°/oo for organic matter (-35°/oo in the Precambrian) and around -25°/oo for the reduced carbon in magmatic rocks] remains an enigma mainly because of the small numbers of analyses of total carbon and gases in critical samples (δ^{13}C measurements are needed from deep-seated rocks, diamonds and kimberlites, fluid inclusions and locations such as basement fractures, kratons, subduction zones, back-arcs, fore-arcs, transform faults and so on).

Methane is a stable form of carbon in the early solar nebula and therefore should have contributed to the primitive Earth. Unlike the primordial noble gases, most of

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* The discussion meeting 'Fossil fuel Biogenic versus non-biogenic' was organized by the Petroleum Geochemistry Group of the Geological Society and held at Burlington House, London, on 7 October 1982

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which are thought to have been lost early in the Earth's history, the other volatiles (H_2O, carbon-containing compounds and so on) were largely retained. Whether one likes Gold's hypotheses or not, and the man confessed to being 'as slippery as oil', he left his audience with many unresolved issues. The possibility that primordial methane still outgasses from the Earth, forms commercially exploitable accumulations and is involved with higher hydrocarbon formation cannot be dismissed out of hand; constructive research and exploration is necessary. □

An enlarged view of intracortical connectivity

from Nicholas V. Swindale

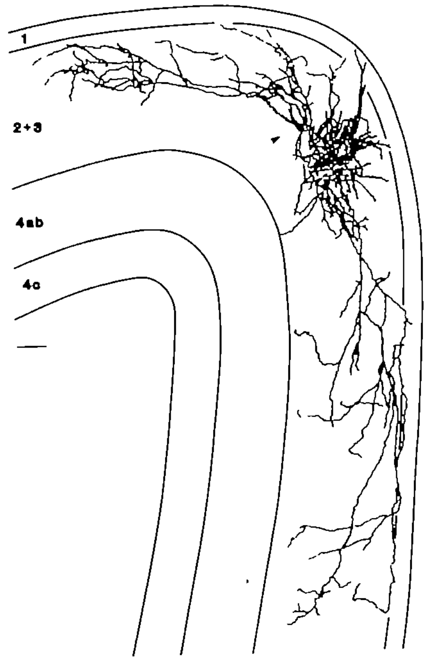
THE conventional view of intracortical connectivity, derived from the results of Golgi[1] and degeneration studies[2], is that lateral connections between nerve cells in the cortex are confined to distances of 1–2 mm or less. However, recent work involving studies of the transport of the enzyme horseradish peroxidase (HRP) following either extracellular[3-5] or intracellular[6,7] injection into the cortex suggests that some intracortical connections may extend over much larger distances. The functional significance of these long-range connections is unclear, but they do seem to be related to some aspect of columnar organization since, like many of the inputs to the visual cortex from other cortical and sub-cortical areas, they are organized into regularly spaced patches with a periodicity of about 1mm or less.

The clearest evidence comes from some experiments of Gilbert and Wiesel[6,7] in which they recorded intracellularly from neurones in the visual cortex of the cat, and then filled them with HRP so that their axonal and dendritic morphology could be reconstructed. Many pyramidal cells were found to have axons that extended laterally over distances of up to 3–4 mm, making periodically spaced clusters of connections (see the figure). The clusters of any one cell may be present in more than one cortical layer, and when they are, they are often in register, suggesting a relationship to some aspects of columnar organization. One reason why this observation has not been made before is that the intracortical axons are often myelinated, and thus fail to impregnate with the Golgi stain; another is that the patchy distribution of axon terminals is often only clearly evident when three-dimensional computer graphics techniques are used to rotate the reconstruction of an axon so that it can be viewed from a particular direction.

Another demonstration of what seems to be the same phenomenon comes from some experiments performed by Rockland and Lund[3-5] in which small extracellular injections of HRP were made into the visual cortex of tree shrews and monkeys. The HRP is transported into periodically spaced patches of cells and axons extending for distances of several millimetres in the upper layers of the cortex surrounding the injection site. Interpreting these results in terms of connections between different columns poses something of a problem however. Suppose that the long-range connections only link up columns of the same type — that, for example, columns of vertically orientation-selective cells only make connections with other such columns, and that columns for horizontal and oblique orientations are similarly interconnected. An HRP injection that was small enough to label the projections and inputs of only one such column would show the observed patchy distribution of HRP transport to similar neighbouring columns. But Rockland and Lund believe that their injections should have been big enough to label the connections with not just one such column, but many of them. As different columns occupy complementary regions of tissue, the pattern of HRP transport expected, on this line of reasoning, should have been much more uniform than was observed. Rockland and Lund's interpretation of the patchy HRP transport therefore is that the long-range connections link up only some regions of cortex and not others — something they refer to as a fixed-position connectional lattice.

Other explanations of Rockland and Lund's result cannot be ruled out at this stage however. One possible complication is that the HRP injection sites might be much smaller than they seem. Much more HRP might be taken up from the very small region of damage caused by the injection than from intact cells. Another possibility is that some cells are intrinsically more likely to take up HRP than others, and that these cells have a patchy distribution. This might conceivably be related to differences in transmitter type, or metabolic activity, for which there is evidence of a patchy variation in the monkey[8,9].

Even if these complications can be ruled out, patchy local uptake of HRP from a large injection does not necessarily imply that some regions of cortex possess long-range connections while others do not. Mitchison and Crick[10] have recently proposed some simple and plausible rules of connectivity between orientation columns that could explain the Rockland and Lund result. They suggest that connections between columns are not distributed radially, but are oriented in a direction that is related to the orientation selectivity of the cells that are being connected. As an example of why this might be so, consider Hubel and Wiesel's proposal[11] that complex-cell receptive fields are made by connecting together a number of simple-cell receptive fields. As a complex cell will respond to a line anywhere in a roughly circular receptive field, and the simple-cell receptive field is elongated, it would be necessary to join together a row of simple cells, the row running perpendicularly to the receptive field orientation. In the cortex therefore, the connections between columns would have to be made in a direction at right angles to the orientation preference of the cells in the columns, mapped onto the cortical surface. In the case where the columns form parallel stripes running in a horizontal direction in the visual field (as they do in both cats and tree shrews), vertically selective cells would have axonal fields elongated in the same direction as the stripes, while horizontally selective cells would have axons cutting across the stripes and making patchy connections. Given this pattern of connectivity, one can predict the local distribution of HRP uptake from an injection large enough to label the pro-



A pyramidal cell in layer 2+3 of the visual cortex of the cat traced by intracellular injection of horseradish peroxidase. Some axons extend laterally over distances of up to 3–4 mm.

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jections to and from many adjacent columns. It is perhaps not intuitively obvious what this would be, but computer simulations show that the distribution of label would resemble that observed by Rockland and Lund, the patches having the same spacing as the orientation columns and running in a similar direction.

Mitchison and Crick's hypothesis of course applies to any system of long-range intracortical projections whose direction in the cortex bears some consistent relation to the orientation preference of the cells being connected (for example, to a system of connections designed to produce very elongated receptive fields by joining them end to end rather than sideways on). In this broad sense the hypothesis seems, *a priori*, more likely to be correct than not. Gilbert and Wiesel's anatomical results do provide some support for the idea, since they show that the axonal fields of cortical neurones are often elongated, though exactly how the direction of elongation relates to the receptive field orientation is not yet quite clear.

At this stage it might be better to wait for more experimental results before speculating further on the nature and function of the patchy intracortical connections. One needs to know, for example, how the HRP patches relate to other columnar systems, such as those for orientation and ocular dominance, and whether the patches formed by one cell innervate columns with similar, or different properties from those of the cell itself. It should also be a fascinating exercise to discover whether the patchy intracortical connections follow the same rules that characterize the development of the geniculo-cortical projection, and whether visual experience can similarly alter their morphology. □

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Catch a passing monopole

from John Ficenec and David Olive

THE first international workshop to focus exclusively on both the theoretical implications and experimental techniques relevant to the detection of magnetic monopoles (isolated magnetic charges) was held recently at Wingspread*. More than 80 experts from an enormous spectrum of physics subdisciplines were brought together in Frank Lloyd Wright's last and largest prairie home through the courtesy of the Johnson Foundation. The meeting was called in response to an observation this spring by Blas Cabrera (*Phys. Rev. Lett.* **48**, 1378; 1982) of an event whose interpretation was consistent with the passage of a magnetic monopole.

The observation has not been corroborated either by Cabrera or others. The flux limit placed on the monopole after collecting more data and a refined analysis was 10^{-10} monopoles cm^{-2} sr^{-1} s^{-1}. This flux remains uncomfortably high when compared with the so-called Parker bound (E.N. Parker *Astrophys. J.* **160**, 383; 1970) of 10^{-15} monopoles cm^{-1} sr^{-1} s^{-1} at which galactic monopoles would dissipate the observed magnetic field of the Galaxy faster than it could be regenerated. Theoretical scenarios to resolve this discrepancy and to explain the candidate event were presented and debated. Numerous experiments, many in the development stage, were discussed. The topic remained in a state of flux with numerous theoretical and experimental questions unanswered, but the participants were optimistic that many problems could be resolved within the next few years.

One of the aims of physics is unification — the organization of the forces of nature, nuclear/strong, electromagnetic, weak and perhaps even gravitational, as different facets of a single symmetrical entity. Calculations indicate that the symmetry is only apparent at energies of 10^{14} GeV, and the characteristic differences between forces at ordinary energies arise because of symmetry breaking. Although this theory is elegant, the enormous disparity in scale between 10^{14} GeV and the proton rest mass energy of 1 GeV is unsettling, particularly since the high-energy regime is inaccessible to any conceivable particle accelerator. However, one consequence is that protons will decay (see *News & Views* **299**, 295; 1982) with a lifetime of $10^{31\pm2}$ yr which should be observable in large-volume experiments already set up and running.

Another prediction of the grand unified theories is the existence of magnetic monopoles which elementary text books tell us do not exist (see *News & Views* **277**, 174; 1979). One of these might weigh as much as 10^{16} protons and, hence, be too heavy for production by particle collisions except in the first 10^{-35} seconds of the life of the Universe, if the latter was indeed created in a big bang as is currently believed. These monopoles would be expected to survive unscathed until the present day but their signature was not observed before this year.

Several suggestions were made to reconcile the discrepancy between Cabrera's upper limit and the Parker bound. One was that the monopole was as heavy as the Planck mass — 10^3 times heavier than previously assumed — thereby increasing the Parker limit. Another was that the distribution of monopoles is not uniform in the Universe, clustering, for example, in the galactic haloes near where we reside or near massive objects such as our Sun.

The experimental challenge was seen to be the detection of a monopole flux as low as the Parker figure. Coincidence experiments using superconducting coils in 10^{-8} G with area 10^2 cm^2 and in 10^{-2} –1 G with area 10^2–10^4 cm^2 were described. These detectors are sensitive to the passage of a magnetic charge, whatever its velocity. Large-area (10^6 cm^2) scintillators and Cherenkov counters, such as those used in proton decay experiments, are being adapted, and a 'football field' size (10^8 cm^2) acoustic detector is being planned. Yet another approach attempts to discover monopoles trapped in matter by positioning a superconducting coil under an iron ore plant conveyor belt, thereby monitoring more than a million tons of ore per year.

Due to the effect of the galactic magnetic field the velocity of a free monopole is expected to be less than about 10^{-3} of the speed of light, which is somewhat less than the speed of an electron in an atom. This limits the type of energy-loss mechanisms available to the passing monopole, and the optimization of detector materials depends on a better understanding of the effect of these slow monopoles. The detectors based on superconducting coils neatly avoid these difficulties.

Physics thrives on paradoxes created by interactions between different ideas, in this case the commonly accepted pictures of cosmology and particle physics. Hence, one theoretical challenge was the construction of a combined cosmology and particle physics theory which did not overproduce monopoles. It seemed that an inflationary universe is an essential ingredient at least at very early times (J.D. Brown & M.S. Turner *Nature* **298**, 801; 1982).

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*The 'Magnetic Monopole Workshop' held at Wingspread near Racine, Wisconsin, on 14–17 October was sponsored by the North Atlantic Treaty Organization, the US Department of Energy, the US National Science Foundation and the Johnson Foundation. The proceedings will be published in early 1983 by Plenum, New York.

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Another theoretical challenge was the elucidation of a new effect which could change the previous estimates for proton decay if monopoles were abundant. It has been known since the last century that the relative angular momentum of an electric and a magnetic charge has a term equal to the product of their charge times the unit vector pointing along the line joining them. If the projectile passes straight through the target in a collision, then conservation of angular momentum means that the sign of electric charge must reverse in order to balance the reversal of the unit vector. This means the monopole must absorb two units of charge. In a theory including the strong interaction gauge group, baryon number could also be absorbed by the monopole, thereby catalysing proton decay to give a distinctive experimental signature. ☐

Recent (1981–82) evolution of galactic haloes

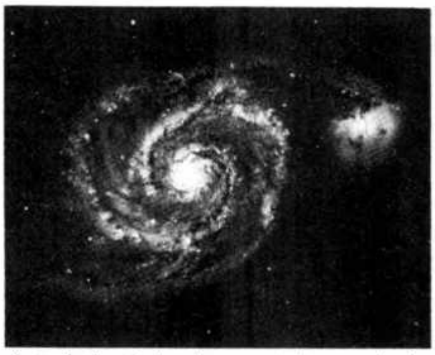
from Virginia Trimble

THE halo of our Galaxy is perhaps a bit older, a bit richer in heavy elements, a bit less massive and undoubtedly a bit more complicated than it was before the recent meeting* of the International Astronomical Union in Greece.

A typical spiral galaxy, whose most conspicuous component is a flat, rotating, armed disk, also has a halo of stars which includes the oldest stars in the galaxy, and which bears at least a passing resemblance to the other major class of galaxy, the ellipticals. Many halo stars are still gravitationally bound in globular clusters of 10^5–10^6 stars in which they formed. There is also a 'scattered field' star population, generally advertized as consisting of fugitives from the clusters.

Among the things we would like to know about the haloes of our own and other galaxies are (1) the ages of the oldest stars (because this sets a significant lower limit to the age of the Universe), (2) their chemical compositions and how they acquired their heavy elements (since, after all, stars formed out of virgin big bang material should be pure hydrogen and helium, and we see no stars like that), (3) whether the globular cluster and field stars are really part of a single population, (4) whether the dynamical structure of haloes is similar enough to that of elliptical galaxies to suggest a common origin (this would considerably simplify the difficult problem of making galaxies out of lumps in the early Universe) and (5) whether there is anything in the haloes besides the stars we see, and, if so, how much of what (because significant mass there will affect our estimate of the average density of the Universe and so its future evolution). New evidence bearing on these questions appeared at the meeting and in recently published papers.

How old are the stars? Rather older than is entirely convenient! The standard method of measuring the age of a star cluster is to compare the positions of the stars in a graph of brightness against surface temperature or colour (HR diagram) with calculated versions of what those positions ought to be as a function of the age of the cluster stars (and, unfortunately, their chemical composition and other variables also). The integrated brightness and colour of a cluster can be used somewhat similarly. These methods have yielded values of 7–25×10^9yr in the past. R. Cannon (Royal Observatory, Edinburgh) and B. Carney (University of North Carolina) reviewed eight more-or-less independent recent applications that concur on ages of 15–18×10^9 yr, at least for the metal-poor clusters. If stars this old are to be fit into a standard Friedmann model of the expanding Universe, then the Hubble constant, H_0, must be less than about 65 km s^{-1} Mpc^{-1}; and if H_0 is to be $\gtrsim 50$, then the Universe must be a low-density (ever-expanding) one as well. According to Carney, the likely errors go in the direction of making these ages, if anything, underestimates. On a related topic, S. Faber (Lick Observatory) discussed evidence from UV photometry of elliptical galaxies indicating that star formation in them also stopped some billions of years ago (though not necessarily so many as 15).



A typical spiral galaxy contains two basic components: a flat, rotating armed disk and a spherical halo of stars which, at their most concentrated, form the central bulge of the disk.

What is the chemical composition of the halo stars? It varies. There has been agreement for some years that the more metal-poor of the globular clusters have about 1 per cent of the solar heavy-element abundance. At the other end of the scale, there has been a continuing discrepancy between photometric studies, which found the most metal-rich clusters to come within a factor of three or so of solar abundance (like the oldest disk stars), and spectroscopic studies, which found much lower abundances. G. Gustafsson (University of Uppsala) drew attention to several sources of uncertainty in both methods, and reported spectroscopic work by M. Bessell (Mt Stromlo Observatory) that yields abundances close to the photometric (higher) values. If this is the right answer, then evidently chemical processing (birth and death of stars) continued in the halo until the remaining gas collapsed down into a disk and began forming stars there. This is rather easier to understand than a picture in which there is a large gap in composition between the youngest halo stars and the oldest disk ones.

Is there a single halo population? Apparently not. The field and globular cluster stars seem to have the same dynamical characteristics, ages and range of metal abundances, but there are detailed differences. R. Kraft (Lick Observatory) suggested that the field stars have, on average, smaller amounts of nitrogen, larger amounts of carbon and a narrower range of abundances of both than do cluster members. This implies either that the field stars are less highly evolved (less thoroughly mixed), meaning that they are younger or less rapidly rotating or something, or that the cluster members have been more thoroughly contaminated by heavy elements made in supernovae in the clusters themselves. Differences between field and cluster stars in the ratio of barium to iron[1] at least weakly support the first, evolutionary, sort of hypothesis. A difference between cluster and field stars in the maximum metal abundance to be found among RR Lyrae and type II Cepheid (pulsating variable) stars is not easily explained by either mechanism[2]. Thus, if the field stars have merely escaped from the clusters, they have not done so at random.

Are elliptical galaxies and the spheroidal parts of spirals the same sort of thing? Again, apparently not. The general shape and ranges of compositions and ages are similar, but there are significant differences. Spiral bulges (the central part of the spheroidal component) have fewer globular clusters per unit luminosity than do ellipticals[3]. This could only be fit into a unified model if clusters were somehow torn apart by the process of disk formation, which seems unlikely. In addition, ellipticals typically show a steeper gradient of composition, from metal-rich at

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*The meeting was held in Patras, Greece on 16–26 August 1982.

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the centre to metal-poor at the edges, than do spiral bulges[4]. This might just mean that chemical evolution operated longer or more efficiently in them. But, finally, well studied haloes show overall net rotation[5], which is not found in most elliptical galaxies and could not very well have been introduced after galaxy formation was complete.

Finally, what, if anything, is in the haloes besides the stars we see? Probably, though not certainly, up to 90 per cent of the mass of the Universe. J. Ostriker (Princeton University) reviewed observational evidence of measured galactic masses that increase roughly linearly with the radius out to which the measurement is made, from the inner 10 kpc sampled by rotation curves, through the 20–100 kpc range sampled by orbits of globular clusters and satellite galaxies, out to the 1–3 Mpc sampled by dynamics of rich clusters (see ref. 6 for another recent summary).

The implication is that a typical large galaxy has associated with it about 10^{12} $M_\odot$ (mass of the Sun) in some form, of which we can see only about 10 per cent in disk and halo stars. D. Schramm (University of Chicago) reviewed the arguments indicating that the invisible material is quite likely to be in the form of exotic particles such as non-zero-rest-mass neutrinos, photinos, or gravitinos. This evidence is largely negative — adequate amounts of ordinary matter could not have escaped detection, either directly or through their influence on big bang synthesis of helium and deuterium.

But two noteworthy arguments against extensive invisible mass, at least in some galaxies, surfaced at the meeting. First, J. Hunter (University of Florida; see also ref. 7) presented a rotation curve for the barred spiral NGC3992 showing 2×10^{11} $M_\odot$ within the inner 19.7 kpc (on the de Vaucouleurs 'short' distance scale) and velocities of satellite galaxies around 3,992 indicating at most 4×10^{11} $M_\odot$ within 59 kpc, so that *M(R)* must increase much less steeply than linearly with *R*, and might stop increasing entirely beyond about 20 kpc. The most relevant response to this is that other galaxies with satellites do show rising *M(R)*, and that even HI profiles (roughly, rotation curves) yield masses of order 10^{12} $M_\odot$ for some galaxies, when the data extend out far enough, as witness the line widths near 800 km s^{-1} for NGC5635 and IC4553 (ref.8).

Second, A. Kalnajs (Mt Stromlo Observatory) showed results of his calculations of the rotation curves produced by various sorts of mass distributions. The result most impressive to his audience is that it is quite possible to get a flat rotation curve, in which velocity versus radius does not drop off at large radius, without invoking a massive, unseen halo component. The secret is a model in which halo and disk have the same constant ratio of mass to luminosity (3–7 in solar units for the galaxies he fit) out to the last measured point, where both end sharply, so that we cannot measure the rotation curve out where we would expect velocity to be dropping with increasing radius in a kepplerian way. The four galaxies fit in this way have data extending out to 5,8, 12 and 25 kpc from their centres; and the fits do not much constrain what is going on outside that. The mass distributions may, indeed, end sharply as in the models or may continue out to 100 kpc or more without distorting the rotation curve near the centre. Which is right must be determined from data that probe further out, such as those from satellite galaxies, binary galaxies and rich clusters, invoked by Ostriker and others in support of massive haloes. Many participants nevertheless came away feeling that the evidence for massive haloes had largely disappeared. This is, perhaps, a natural over-reaction to having had the arguments in favour presented so vigorously at so many previous meetings! ☐

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Human monoclonal antibodies

from Karol Sikora and A. Munro Neville

Now that the application of monoclonal antibodies to the diagnosis and treatment of human disease has become the major goal of many investigators, a limiting factor is the availability of human rather than mouse or rat monoclonals. Human monoclonal antibodies are particularly needed for administration to patients for diagnosis and therapy in order to minimize the problems of administration of a foreign animal protein, with the risks of anaphylaxis and the clinical manifestations of immune complex formation, as well as abrogation of the antibody's effects.

The short history of human monoclonal antibody production has been fraught with technical difficulties but several novel systems now hold out promise. Initial attempts used available mouse and rat myeloma lines for fusion[1-3]. Unfortunately the resulting hybridomas preferentially lose human chromosomes, eventually eject the chromosomes coding for immunoglobulin production and thus lose the ability to produce monoclonal antibodies. Although early and repetitive cloning of the hybrids can reduce the shedding of human chromosomes, the development of more stable human–human hybridoma systems seems desirable.

Accordingly several groups have developed human systems from which hybridomas may be created. Unfortunately many human myelomas are difficult to grow in tissue culture and have low growth rates and poor immunoglobulin secretion. Nonetheless, certain lines have been 'adapted' to grow in culture in genetic selection conditions in which the growth of the myeloma is suppressed but the hybridomas continue to propagate. There have, however, been difficulties with mycoplasma infection in myeloma stocks. Indeed many investigators have been unable to obtain any hybridomas at all with lines donated by certain laboratories, causing considerable controversy.

The use of several human myeloma or lymphoblastoid lines was discussed at a recent workshop in Cambridge held under the auspices of the Ludwig Institute for Cancer Research (LICR). The ideal properties of such a cell system include high fusion frequency, high cloning efficiency, the ability to grow rapidly in non-stringent serum conditions, no secretion of myeloma or lymphoid cell immunoglobulin, and yet the production of large amounts of immunoglobulin after fusion to suitable lymphocyte donors. M. O'Hare (LICR, London) described his experience with the cell line LICR-LON-HMy2 (ref.4) — a derivative of the human 'myeloma' ARH77 (ref.5). This lymphoblastoid line expressing Epstein–Barr virus (EBV) antigens appears to be the best available system. Reasonable fusion frequencies have been obtained from a variety of human lymphocytes but the immunoglobulin secretion rate is low at 0.2–8.0 µg ml^{-1} in 24 h.

K. Sikora (LICR, Cambridge) reported that use of the same line. Intra-tumoral and regional node lymphocytes from the tumour-draining nodes of 180 patients resulted in the successful production of new immunoglobulin-producing hybrids in 24 cases[6]. When these were screened for antitumour antibody activity, weak binding was detected in only nine hybridoma supernatants. More success was achieved when fusing intra-tumoral lymphocytes from patients with malignant glioma, and three monoclonal antibodies to lung cancer were obtained by fusing lymphocytes from intra-thoracic lymph nodes removed at the time of surgical

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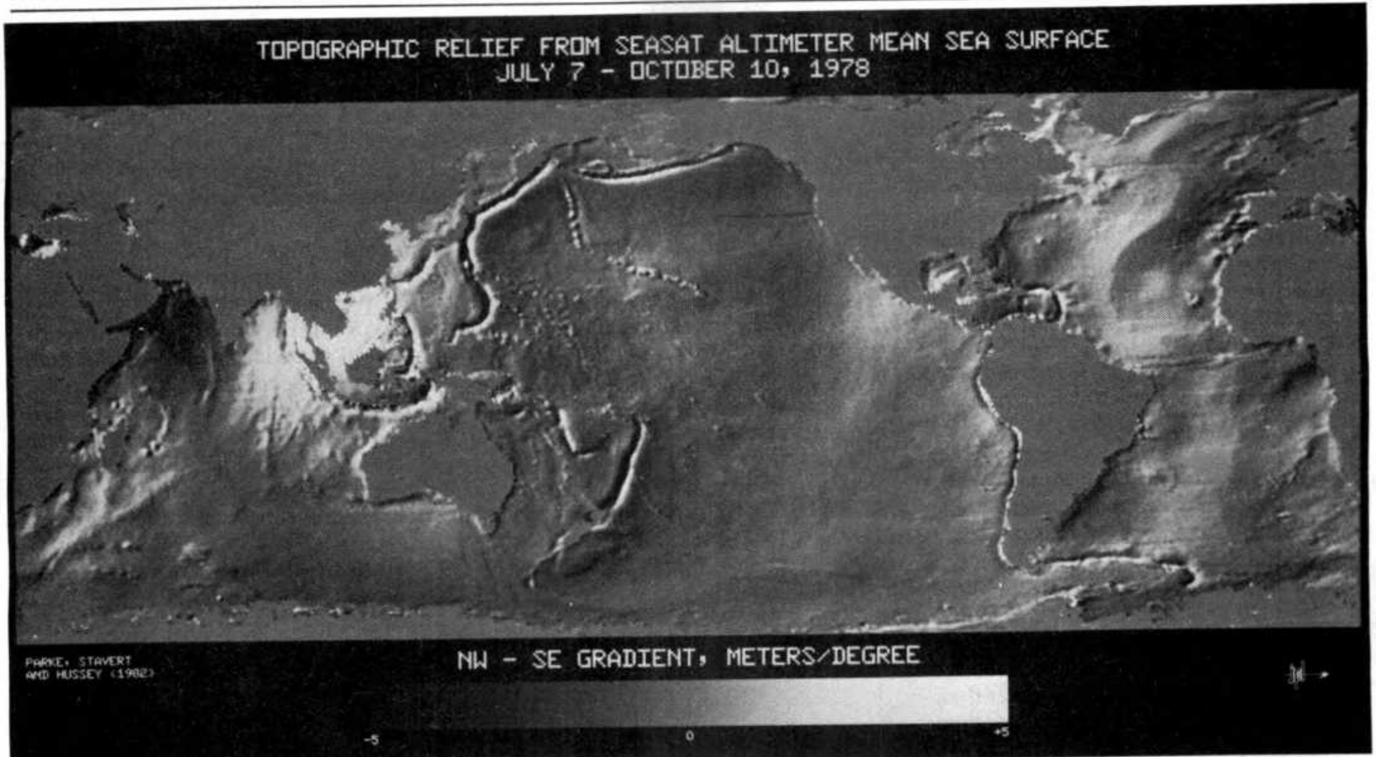
TOPOGRAPHIC RELIEF FROM SEASAT ALTIMETER MEAN SEA SURFACE
JULY 7 - OCTOBER 10, 1978
PARKE, STAVERT AND HUSSEY (1982)
NW - SE GRADIENT, METERS/DEGREE
-5
0
+5

THE gravitational signature of tectonic features can be clearly seen in the topographical relief maps of the world's ocean surface shown above. Data from the Jet Propulsion Laboratory's Seasat oceanographic satellite were used to produce the map after processing to emphasize steep, small-scale features that tilt towards or away from the north-west.

The Mid-Atlantic Ridge and the trenches along the west and north-west margins of the Pacific Plate are clearly visible, as are the chain of Hawaiian seamounts.

The extent to which a seamount affects the local gravity field depends on the age of the underlying crust when the seamount was formed. If the seamount formed on young flexible crust, the crust deforms around the seamount, compensating the effect of the seamount's extra mass. Seamounts formed on older, more rigid crust are not compensated in this way, resulting in a gravitational signature that is more apparent at the sea surface. For use of similar GEOS-3 altimetric data to investigate sea basin formation see *Nature* **299**, 117; *News and Views* **299**, 104; 1982. ☐

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treatment[6]. Again, the secretion rate of patient-derived immunoglobulin was low. None was tumour type specific.

Coté (Sloan Kettering Institute, New York) described his work involving over 80 fusions with three different hybridoma systems — SK007 (which had been rendered free from mycoplasma infection[7]), LICR-LON-HMy2 and NS1, the standard mouse myeloma[8]. Immunoglobulin secretion rates varied from 1 to 100 μg ml^{-1} with no consistent differences between the three fusion systems. Early cloning and stringent culture conditions seemed to be important prerequisites for success with inter-species hybrids if continued production of human immunoglobulin for long periods was to be achieved. Some human antibodies with interesting specificities to tumour cytoskeletal components were demonstrated by elegant immunohistological studies using human tumour lines. Using the lymphoid cell lines UC729-6, I. Royston (University of California, San Diego) was able to produce monoclonal antibodies which reacted with various cancer cell lines, but not normal lymphocytes, granulocytes, red cells or fibroblasts. These antibodies, although binding weakly to a variety of tumour cells, were in no way tumour specific. S. Clark (University of Glasgow) used diethyl pyrocarbonate[9] in concentrations near the mean lethal dose for myeloma cell lines as a selection mechanism for cells whose enzyme components were complemented after fusion. This allowed the outgrowth of hybridomas. He described work using the human myeloma RPMI 8226 and a sub-line of the EB4 myeloma — SJR22C. Again, there were problems of low fusion frequency and poor cloning efficiency.

Perhaps most interesting was D. Crawford's (Imperial Cancer Research Fund, London) account of making monoclonal anti-influenza and anti-rhesus D antibodies by infecting peripheral blood lymphocytes from donors with EVB. Several groups have tried this technique over the last few years without much success, the main stumbling block being the cloning of the EBV-transformed lymphoblast. Using a combination of *in vitro* stimulation by immunizing antigen and early cloning by limiting dilution, Crawford was able to demonstrate that not only could useful antibodies be produced but also the concentration of immunoglobulin produced was at least equivalent to the best human hybridoma systems now available. The necessary ingredients in the recipe for success are still unclear. Several groups are investigating the possibility of initial EBV transformation, selection of cells with the required antibody specificity and subsequent immortalization by cell fusion in a hybridoma system.

We are still unfortunately plagued by technical problems in the production of human monoclonal antibodies, not least in discovering ways of collecting those lymphocytes that produce antibodies with the specificities being sought for different experimental purposes. It is too early to know whether all the current efforts will result in biologically interesting molecules with fundamental and clinical potential or just additional reagents with little difference from other available rodent monoclonal antibodies that can be obtained with significantly less effort. Nonetheless, the prospect of the former result will stimulate many groups to study and develop human hybridoma systems in the near future. The outcome could be exciting. ☐

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The recovery of Halley's Comet

from David W. Hughes

AMERICAN and European astronomers have been competing with each other to be the first to sight Halley's Comet. It was last seen at the end of May 1911, heading away from the Sun and passing below the orbit of Jupiter. It reached aphelion in 1948 and has been racing back towards the inner Solar System ever since.

The Americans have won. *International Astronomical Union Circular*, number 3737, reported that D. Jewitt, G. Danielson, J. Gunn, J. Westphal, D. Schneider, A. Dressler, M. Schmidt and B. Zimmerman recovered the comet on the night of 16 October 1982. They used a charge-coupled device at the prime focus of the 5.1 m (200 inches) Hale telescope at the Palomar Observatory, California. Charge-coupled devices are much more sensitive than photographic plates and with the 5.1 m telescope can detect objects fainter than 25th magnitude, some 50 times fainter than the photographic limit. Five 8-min exposures were taken through a broad-band blue filter. An object was detected near the expected position of the comet, moving in the expected direction at the expected velocity. It had a magnitude of 24.3±0.2 (a change of unity in magnitude is equivalent to a factor of 2.5 in brightness, so Polaris the Pole Star is 7×10^8 times brighter than Halley's Comet at present). Confusion with an asteroid is regarded as extremely unlikely. The recovery was confirmed by M. Belton and H. Butcher who obtained accurate cometary coordinates on the nights of 18 and 20 October using a cryogenic camera and charge-coupled device on the 4 m Mayall telescope at the Kitt Peak National Observatory (*IAU Circ.* no. 3742). Their report of the unsuccessful search during winter 1981–82 can be found in *Nature* (**298**, 249; 1982).

No coma was detected, but this is hardly surprising because the surface temperature of the nucleus is expected to be about 120K. The recovery brightness indicates that the nucleus of the comet, assuming that it has a geometric albedo of 0.05, is about 9 ± 1 km across.

Halley's Comet, now termed 1982i, because it is the ninth new or recovered one this year, is in the constellation of Canis Minor. On 16.47569 October it was at a Right Ascension of 7 h 11 min 01.9 s and a declination of $+9°33'03''$, putting it only 0.6 s west of the position predicted by D. Yeomans (*The Comet Halley Handbook*, JPL 400-91 1/81; 1981). The comet is early and will pass perihelion some 8.6 h before the expected time of 9.66128 February 1986. At recovery Halley's Comet was 11.04 AU away from the Sun, so it has broken two records — it is the most distant comet ever seen, beating Stearn's Comet (1927 IV) by about 1 AU, and the faintest comet ever recorded.

The fact that Halley has been recovered 3.3 yr before perihelion passage is also amazing. At its 1910 return it was first seen on 11 September 1909 about 7.5 months before perihelion by Max Wolf, who used a one-hour photographic exposure with the 72 cm Heidenberg reflecting telescope. It was then at the outer edge of the asteroid belt about 3.4 AU from the Sun and of magnitude 16. It was 10 arc min from the predicted position and the previously predicted perihelion passage time was 3.03 days too early.

The Europeans were also first at the return before that. Dumouchel, the director of the Collegio Romano Observatory, obtained the first telescopic view of the comet on the morning of 6 August 1835, 3.3 months before perihelion passage. At that time the comet was 17 arc min in declination and 7 arc min in Right Ascension away from the predicted position, giving a predicted perihelion passage time 5 days too early.

So it seems that congratulations are in order. Yeomans' positional predictions have been proved to be extremely accurate, even though the comet is three times fainter than estimated. And the team who recovered the comet have pushed astronomical techniques to the limit and succeeded. All the scientists preparing space experiments for the 1986 perihelion passage of Halley's Comet can breathe a sigh of relief too. It is where it was expected to be, coming towards us at a speed of about 11 km s^{-1}; they have just got 8.6 h less time to get ready. ☐

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Confident carbon calibrations

from D.D. Harkness

SEVERAL proposals of fundamental importance to the confident interpretation of ^{14}C data were put forward at the 11th International Radiocarbon Conference[*]. Of major consequence was a plan to revise the constants and format used in the calculation and reporting of conventional ^{14}C years BP. In the event, the plan was overwhelmingly rejected in favour of the *status quo* — definition of the time scale by use of a half life of 5,570 ± 30 yr and the setting of AD 1950 as the zero age. Similarly, it was accepted that the expression of analytical uncertainty should remain as the individual laboratory's best estimate of one sigma statistical confidence in age measurement. In contrast, no agreement could be reached on the question of a standard procedure for isotopic normalization in the calculation of marine shell ages.

Routine accuracy in ^{14}C measurement was recently assessed in various laboratories on the basis of tree-ring dating (International Study Group *Nature* **298**, 619; 1982) and a review of the results suggested the need for a more frequent exchange of samples between laboratories and the uninhibited publication of such results. Practicing laboratories also welcomed the opportunity for collective assessment of the new reference material (1980 oxalic acid) issued by the US National Bureau of Standards. Following appraisal and discussion of its isotopic ratios, as measured relative to the established (1957 NBS oxalic acid) standard, the issue of the replacement standard was endorsed but it was noted that an accompanying statement on the isotopic enrichment factors proposed for routine dating work was desirable.

Causal mechanisms for variations in natural ^{14}C concentration received considerable attention and a consensus emerged that the established long-term and high-frequency (wiggle) variations in ^{14}C production rate primarily reflect changes in geomagnetic field intensity. Solar and climatic effects cannot be entirely precluded from the available tree-ring/^{14}C record but they are certainly small — no clear correlation is evident, for example, between the existing carbon isotope (tree-ring) record and historically recorded climate (M. Stuiver, University of Washington). Nevertheless, a measurable impact on the atmospheric ^{14}C inventory could be expected from the marked changes in the dynamics of the global CO$_2$ system during the glacial to postglacial transition (H. Oeschger, University of Berne). Beyond the range of dendrochronological comparison, parallel ^{14}C and ^{230}Th ages from cave deposits, which are concordant during the Holocene, indicate a higher ^{14}C content in the Peniglacial atmosphere — a major ^{14}C

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[*] The 11th International Radiocarbon Conference was held at the University of Washington under the sponsorship of the National Science Foundation, the Weyerhaeuser Company and the Quaternary Research Centre, Department of Geological Sciences and Department of Physics, University of Washington The conference proceedings will be published in *Radiocarbon* 25 (1983)

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fluctuation at about 29,000–31,000 BP is again attributable to variations in global magnetic field strength (J. Vogel, University of Pretoria).

Ongoing attempts to determine the precise secular pattern of past ^{14}C variations mainly make use of the opportunity provided by tree-ring data for 'absolute' calibration of ^{14}C ages. Significant progress has been made in compiling continuous ($\sim$8,000 yr) chronologies from Irish and central European oak deposits with only a few gaps remaining to be bridged in each sequence (J. Pilcher, University of Belfast). Although numerous high-precision ^{14}C measurements with good agreement between laboratories are already completed from these oak deposits, an attitude of caution prevailed over early publication of the 'European oak calibration' as a more precise, but as yet partial, alternative to the re-evaluated Bristlecone pine record (Klein, J., Lerman, J.C., Damon, P.E. & Ralph, E.K. *Radiocarbon* **24**, 103; 1982).

Several papers *en bloc* served to confirm the continuing role of $^{14}C/^{12}C$ variations, both natural and man induced, in studies of the contemporary environment. Appreciable coverage was given to the use of ^{14}C measurements, in conjunction with allied geochemical data, to establish the recharge, transport and carbon exchange characteristics of regional groundwater systems. The continued interest in ^{14}C as a quantitative tracer for mass transfer highlighted the importance of the deep ocean as the ultimate sink for fossil CO_2; however, the net uptake of this anthropogenic excess must lead eventually to the increased dissolution of sedimentary calcite (T. Peng, Oak Ridge National Laboratory). Akin to its value in identifying urban sources of atmospheric and freshwater pollution, the sensitivity of ^{14}C measurement can contribute to the direct evaluation of localized gaseous dispersion patterns from nuclear installations (R. Otlet *et al.*, AERE, Harwell).

Awareness of intrinsic contamination in sample materials selected for conventional dating studies was a recurrent theme. Organic-poor sediments, often preferred for their more lucid geomagnetic signature, are particularly susceptible to the 'ageing' influence of allochthonous carbon (J. King, University of Minnesota). Similarly, while ^{14}C analysis of freshwater carbonates can provide interesting geochemical insights, the inferred dates are, in general, suspect (R. Pardi, Queen's College, CUNY).

A comprehensive review of technical progress in the application of accelerator mass spectrometry to natural ^{14}C measurement came from some eleven research groups active in the field. In essence, while the great promise of a revolutionary breakthrough in ^{14}C dating method seems ever closer, it has yet to be fulfilled. The routine preparation of suitable and isotopically homogeneous targets, together with the need to attain improved accelerator stability during and between sample runs, are outstanding constraints to the achievement of an analytical precision comparable with that established in radiometric counting. However, an incentive to continued development was the recent achievement of about 5 per cent analytical precision (equivalent to ± 400 yr dating uncertainty) in $^{14}C/^{13}C$ measurement using milligramme-sized carbon samples (D. Donahue, University of Arizona). By present comparison the proven, if less instrumentally demanding, application of this analytical approach in natural ^{10}Be measurement represents an immediate and exciting prospect for Quaternary research.

From shilling prisms to digitized images

from L.T. Sharpe

SELDOM has the field of colour vision been more stimulating than it is today when new techniques allow us to explore what for centuries has been mere matter for speculation. Modern methods and historical contributions were jointly emphasized at a recent international meeting* on colour vision held in Cambridge — a fitting place to bring together colour scientists because the problems of colour have exercised many of the university's most distinguished physicists and physiologists, including Newton, Young, Maxwell and Lord Rayleigh. Indeed, for many at the conference, the beginnings of modern colour science were betokened by a holograph notebook (lent for display by the Fitzwilliam Museum) in which Newton recorded his purchase in 1667 of three prisms for three shillings.

The meeting covered an unusually wide range of topics from microspectrophotometry of visual receptor cells to the phenomenological constancy of coloured surfaces illuminated by spectrally very different sources.

Perhaps the most impressive advances have been made at the very beginning of the visual pathway, in studies of visual transduction in single primate photoreceptors by Nunn and Baylor of Stanford University that have just been reported in *Nature* (**299**, 726; 1982). Individual outer segments of rods from cynomolgus monkeys (*Macaca fascicularis*) were sucked into micropipettes of small (2μm) orifice diameter so that the reduction in membrane current that occurs when light stimulates the outer segment could be measured. Transverse stimulation of the outer segments by spectral lights over the range 400–720 nm produces a spectral sensitivity curve with a peak at 491 ± 3 nm. An interesting discrepancy is thus revealed — the result agrees well with psychophysical measures of human scotopic sensitivity (if corrections are made for lens absorption and pigment self-screening) but not with microphotometric absorption measurements, where two independent groups find a peak value near to 500 nm. The difference recalls that seen between measurements of rhodopsin spectral sensitivity *in vivo* and in digitonin extract.

Only very preliminary results using the new technique are available for cones and for detailed information about their sensitivities we must rely mainly on microspectrophotometry. Recent technical refinements — transverse measurements that allow good optical isolation of the outer segment and yield reasonably high optical densities; digital computers that streamline data-gathering and analysis; and procedures for partial glutaraldehyde fixation of the retina that help maintain the structural integrity of the cone outer segments — have greatly increased the accuracy of microspectrophotometric data. Two separate groups, one in London (J. Bowmaker, H. Dartnall and J. Mollon), the other at Wood's Hole, Massachusetts (E. MacNichol Jr, J. Levine, R. Mansfield, L. Lipitz and B. Collins) are making precise measurements of the photopigments of individual rods and cones, using both fresh material and lightly fixed isolated retinae. The two groups substantially agree about the shapes and locations of the λ_{max} of the major classes of pigment in the Old World monkey species (rhesus and cynomolgus). But residual differences exist concerning the properties of the short-wavelength-sensitive cones. The Wood's Hole group finds a longer λ_{max} for the rare short-wave cones of the cynomolgus monkey (429 ± 4 nm; $n = 8$) than does the London group (415 nm; $n = 2$). (Neither group finds any short-wave cones in the rhesus monkey.)

A new question is whether, within the normal human population, there are slight

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*An international conference on colour vision was held at the University fo Cambridge, 23–27 August 1982 The primary sponsor was the Scientific Affairs Division of NATO; additional support was provided by The Royal Society, The Welcome Trust and the US Army Research Institute for Behavioral and Social Science

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variations between observers in the spectral positions of their photopigments. Factor analysis of Stiles and Burch's 10-degree colour-matching data suggests that there are (D. MacLeod and M. Webster). The standard deviation of peak absorption wavelength (for a given receptor class) among individuals is estimated between 1 and 1.5 nm. Human microspectrophotometric data suggest a somewhat larger variance. For instance, the London group showed that two 'normal' clinical patients (normal with respect to Rayleigh anomaloscope and Farnsworth-Munsell 100-hue results) had an 8 nm (553 and 561 nm) difference in the $\lambda_{\max}$ of their long-wavelength pigment sensitivities. A similar difference in long-wavelength sensitivity was found between the patients' field sensitivity ratios calculated at 555 and 650 nm. Even larger variations in the $\lambda_{\max}$ of the middle- and long-wave cones are found for the squirrel monkey, *Saimiri sciureus*. These too are well correlated with differences in behaviourally measured colour vision (G. Jacobs, University of California).

A new research tool is provided by digital computers that can control the display of digitized images on a colour television monitor. The way in which a given digital value is translated into a particular colour can be controlled and rapidly altered so that a stimulus can be moved in any direction in colour space. Despite some technical problems arising from instability, non-uniformity and flicker (W. Cowan, National Research Council, Ottawa and R. Rodieck, University of Washington), raster-based colour stimulators can be impressively used (1) to simulate changes in the illumination of complex coloured scenes so that the visual computations underlying colour constancy may be analysed (J. McCann and K. Houston, Polaroid Corporation) and (2) to quantify the chromatic properties of neurones of the macaque lateral geniculate nucleus in terms of the secondary-stage processes or 'channels' of colour vision (A. Derrington, J. Krauskopf and P. Lennie, University of Sussex).

This last is very important because it is commonly assumed that visual information is transmitted centrally by two parallel and independent 'channels' — the achromatic and the chromatic. Although electrophysiology often appears to support this post-receptoral dichotomy, careful inspection of the properties of colour-coding retinal ganglion cells shows that their colour opponency is not a fixed characteristic, but rather varies with temporal (duration and frequency) and spatial (size and position) properties of the stimulus (E. Zrenner, Bad Nauheim). For instance, at low temporal frequencies (5 Hz), red-green colour opponent cells have two sensitivity maxima, one for excitation (say +R) and one for inhibition (say −G) with a broad neutral zone in between. At higher frequencies (30–33 Hz) colour opponency is lost; the spectral sensitivity function becomes broadband and single-peaked, resembling that of luminance-detecting cells. The increase in sensitivity in the midspectral region seems to be based on a phase shift between the centre and surround responses which turns an antagonism into a synergism.

100 years ago

Goolden's dip-circle, claimed to make readings correct to within 10 minutes of arc. From *Nature* 27, 128, 7 December 1882.

These results beg comparison with psychophysical experiments. Here too the simplistic view that it is possible to isolate 'channels' corresponding to two classes of neurone in the visual pathway is being challenged. The X-cells of the retina, whose small receptive fields predominate in the central fovea, are usually assumed both to code colour and to mediate resolution of acuity targets. When acuity is used as a criterion for equating the luminances of different colours, mixtures of such lights are photometrically additive. Additivity, in the conventional view, is a property of the luminance channel of psychophysics while the opponent chromatic channels are assumed to account for the nonadditivity of direct brightness matching and threshold measurements. How can the opponent red-green cells be nonadditive for threshold targets (detection of low spatial frequencies) and additive for acuity targets (detection of high spatial frequencies)?

All these complications suggest that colour and spatial vision may not be easily separable into discrete, processing channels. Indeed, even if separate channels exist for achromatic and chromatic vision, they may not be independent — luminance and chromaticity certainly interact in grating detection tasks. The interaction is not, however, symmetrical: colour masks luminance much more profoundly than luminance masks colour (K. De Valois and E. Switkes, University of California). The masking cannot be due purely to local (retinal) interactions because it is independent of relative test-mask phase. It must, therefore, occur at a level at which global chromatic and spatial frequency characteristics have been encoded.

Colour processing in the cortex is an area of hotly contested opinion. Two major controversies were discussed at the meeting. The first concerned whether particular parts of the primate prestriate cortex are specialized for colour analysis, as has been proposed by S. Zeki (*Nature* **284**, 412; 1980). Some researchers recording in anaesthetized and paralysed vervet monkeys find colour-coding cells dominating that part of area V4 lying on the lateral part of the anterior bank of the lunate sulcus (V. Andersen, C. Guld and O. Sjo, University of Copenhagen). Fewer are found in V2, almost none in V3. The colour-coding cells are often narrowly tuned for orientation (± 10 deg) and their spectral responses depend strongly on both the wavelengths of the adapting fields and the luminance of the stimulus. About half respond to white on a white background (usually a defining characteristic of luminance-coding cells). Other researchers, however, find few colour-coded cells in these areas when recording from alert, behaving rhesus monkeys (J. Krüger, B. Fischer and R. Boch, Freiburg I. Br.). Only about 10 per cent of the cells respond better to one or another colour. Rather they seem to have strong extraretinal inputs that are activated before and during visually guided eye movements. It may be, therefore, that in anaesthetized and paralysed animals extraretinal inputs cannot be activated and the excitability of the prelunate cortex depends only on cells with pure efferent sensory inputs originating from the retina. Thus for such 'unnatural' conditions the prelunate cells may merely appear to be colour biased.

The second controversy is whether colour cells in the primate striate cortex are confined to columns extending vertically from the cortical surface to the white matter (see also *News & Views* **300**, 220; 1982). In the rhesus monkey, the columns are identified as slabs or stripes which are 100–250 μm wide and of indeterminate length (C. Michael, Yale University). Within a column, all neurones respond exclusively to monochromatic stimuli but differ in their receptive field organization, axis orientation, or eye preference. In the vervet monkey, however, there is no evidence for such colour columns (J. Krüger and M. Bach). Experiments in which 0.8×0.64 mm^2 arrays of 30 parallel microelectrodes are used reveal non-colour-coding neighbouring sites in one cortical layer (say IVc) located directly above colour-coding sites in another layer (V). Such differences may be species dependent or may suggest that instead of colour columns encompassing the whole cortical thickness, independent columnar systems exist for the infra- and supragranular layers. Before any more progress can be made on this issue, a consensus is needed on how to classify cells as colour coding. Some researchers classify cells as colour coding if they respond to coloured, but not white, stimuli; others if they respond differently to two colours irrespective of other stimulus parameters; and still others if they show a large difference in response strength to two colours for at least one out of several stimulus configurations. □

REVIEW ARTICLE

Origin, nature and world climate effect of Arctic Ocean ice-cover

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During the Cenozoic, an open water Arctic Ocean changed to the modern permanently ice-covered condition. Significant world climate modification accompanied this change but the precise role of the Arctic Ocean in major Pleistocene climate events is controversial. The present ice-cover averages 3 m thickness but there are theories that during the Pleistocene it was Antarctic-like, several thousand metres thick. The time of origin of the ice-cover is placed as young as 0.7 Myr ago and as old as the middle Miocene. Any theory concerned with origin, nature and climatic response of the Arctic ice-cover must consider the ocean's sediment record.

THE modern Arctic Ocean above 75° latitude has modest circulation with Pacific and Atlantic Oceans but is largely ice-covered during most of the year (Fig. 1). The detailed study necessary to understand Arctic Ocean history is not complete, in part, because of the logistical difficulties of working in an ice-covered ocean. Limited geophysical, oceanographic and biological studies have been accomplished even in the most inaccessible parts of the Ocean by use of the Arctic's ice as a platform from which to work. More than 500 short sediment cores that include the geological record of the central Arctic for parts of the Cretaceous to Holocene have been recovered from the ocean floor. Study of the sediment, its fauna and flora, provides data on sediment source, rate of sedimentation, water productivity, temperature and salinity and relationship of the Arctic water to the world ocean. In addition, aerial geophysical surveys have added to the Arctic Ocean data base[1]. The limited data assembled from this work is important for understanding elements of geological and climatic development of the present Ocean as well as its role in the tectonic evolution of the Northern Hemisphere[2]. Also, it is apparent that the Arctic Ocean has a significant role in the development of world climates during at least the past 75 Myr and may be an important factor in any future CO_2-induced climate change[3].

During the Cenozoic, world climate changed from one with a low thermal gradient, only about 50% of that of the present, to the modern condition of strong gradients[4]. The oldest known Arctic Ocean sediment is late Cretaceous and it accumulated in an ice-free environment[5]. The absence of an Arctic ice-cover was an important factor contributing to the lower atmospheric and oceanic thermal gradients that allowed temperate climates almost to reach the poles during the Cretaceous[4]. World-wide, these conditions changed sometime following the Cretaceous. The development of polar ice-covers probably dates from the Middle Cenozoic or late Neogene[6] (Fig. 2). The Arctic Ocean sediment record is not complete for this period of time but glacial–marine sediment was accumulating at least by the late Miocene in the central Arctic[7]. Together with Antarctic ice development, an Arctic ice-cover produced a profoundly steeper thermal gradient than had existed during the Mesozoic. This was a factor in the initiation of Pleistocene glaciation and modern climate.

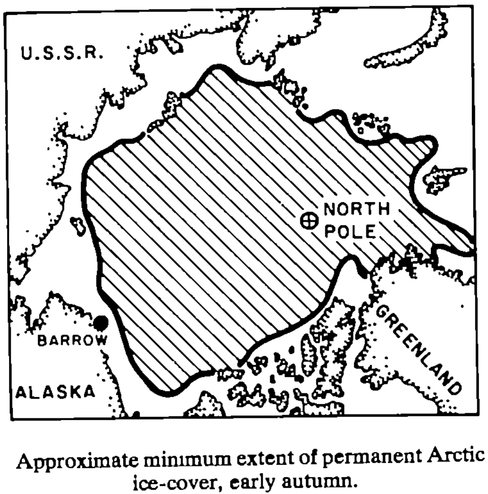

Fig. 1 Approximate minimum extent of permanent Arctic Ocean ice-cover, early autumn.

The evidence for the Arctic's role in the late Cretaceous to middle Cenozoic climate change is fairly well understood. The Arctic sediment record is one of biogenic silica, diatoms and silicoflagellates, probably representing relatively warm upwelling conditions during the late Cretaceous[5]. This was followed by a change to glacial–marine sedimentation for at least the late Miocene to Holocene[7]. The sediment record for the entire late Cenozoic Arctic Ocean indicates that more or less continuous glacial ice-rafting has been the principal sedimentation process. There were times when ice-rafting was very intense, alternating with time of less clastic sedimentation[7]. During these intervals of limited ice-rafting, increased biological productivity is suggested by increase in foraminifera, ostracodes and calcareous dinoflagellates. These data must be considered part of any theory that attempts to explain the nature of the Arctic ice-cover although it has not been used by those who have proposed fluctuating climatic regimes or giant ice-caps for the late Cenozoic central Arctic Ocean. Also while the data on sediment types are unequivocal, the timing of the development of the first Arctic ice-cover as well as the precise sequence of events leading to modern conditions are issues not fully resolved. The 'fine tuning' for resolution of time of initiation of the Arctic ice-cover may not be accomplished until a very complete central Arctic Ocean sediment core is recovered. Meanwhile based on short sediment core study, the discussion of the palaeoclimatologic changes that accompanied the development of the modern Arctic Ocean has produced diverse ideas.

Arctic role in the world climate

The importance of the Arctic Ocean for world climate was recognized by the 1956 Ewing–Donn theory[8]. In this early attempt at an unified explanation for Pleistocene glacial cycles, the Arctic Ocean had a central role. According to the theory, an open, ice-free ocean produced moisture to support the expansion of glaciers. Melting and retreat of glaciers in lower latitudes occurred when an Arctic Ocean ice-cover formed, sealing off the source of moisture and thus affecting glacier

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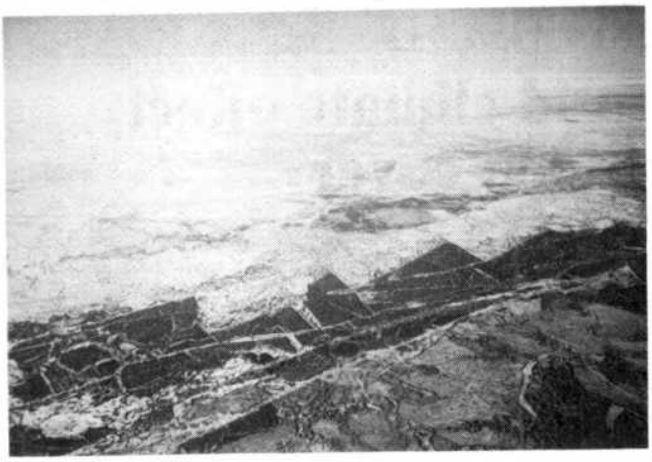

Fig. 2 The modern Arctic Ocean ice-cover. Aerial view showing dark, fine grained sediment laden pack-ice. Irregular pattern of sediment-rich ice and cleaner pack-ice due to fracturing of pack-ice during movement.

growth. In this ingenious theory, glacial and interglacial intervals of the Pleistocene were thought to be highly dependent on the condition of the Arctic Ocean surface.

Confirmation of the validity of the Ewing–Donn theory could be tested in various ways. In the years following its publication, palaeontologists, marine geologists and others gathered diverse data that questioned many of the theory's basic tenants. Using pollen data from the area around the Arctic basin, Colinvaux[9,10] summarized objections to the theory based on ecological constraints of demonstrated floral occurrences. Next, sediments and sedimentation rates in Arctic cores were found to be inconsistent with the theory[11–13]. Foraminifera abundance patterns for the central Arctic during the past 1.5 Myr showed no time of ice-free conditions that could be correlated with glacial or interglacial stages[14]. This raised additional objections to the theory and it was concluded that the Arctic must have remained frozen while lower latitude Pleistocene glacial advances and retreats were in progress[14]. Alternating thickening or thinning of the ice-cover may have occurred during the Pleistocene but the sediment, floral and faunal record seemed to confirm that there was no evidence for an open Arctic Ocean that could be correlated with Pleistocene glacier growth. Thus, the Ewing–Donn theory was untenable.

If the Arctic ice-cover was not cyclic in appearance and disappearance during the Pleistocene, (and it was ice-free in the earliest Cenozoic) the obvious question was the time when the Arctic Ocean first become ice-covered. Also, what were the changes that accompanied the great freeze and what was the condition of the Arctic Ocean throughout the Cenozoic? Although these questions could be answered by study of a continual sediment record for the Arctic Ocean, no DSDP Leg has been planned for the central Arctic and the ice problems are so profound that no similar drilling program is contemplated. However, extensive coring from floating ice-island T-3, produced several short sediment cores and their study produced good data. Most of the cores recovered during the T-3 years (1955–74), some 580 successful casts, were taken as part of heat flow studies by the US Geological Survey (A. Lachenbruch and V. Marshall). After conductivity measurements, sediment and faunal studies of the cores were undertaken at the University of Wisconsin-Madison. In addition several cores were taken by a group at Lamont-Doherty Laboratory (under K. Hunkins) and became the basis for work at Lamont as well as later work at Washington State University. The Arctic sediment cores yielded a wealth of data that were subsequently used in faunal analysis[12,14–24], sediment studies[7,25–28], and became the basis for diverse palaeoenvironmental interpretations[14,29–34]. These environmental interpretations have assumed new importance because of theoretical considerations related to a possible future CO_2 induced world climate change[3]. If a climate change occurs, one of the first effects may be the disappearance of at least the summer Arctic ice-cover[3]. In order to understand the environmental impact of such an event, climatologists have considered the possibility that there was a time in the recent geological past (past 3 Myr) when the Arctic had no ice-cover. If such a time occurred, it could provide an accurate analogue for understanding the change that might occur in the future[3].

There is no uniformity of thought concerning conditions of the Arctic ice-cover during the past 3 Myr. From the various data based interpretations as well as theoretical considerations, three different theories have been proposed to explain the evolution of the modern Arctic Ocean: the (1) fluctuating regime and (2) steady state theories are based on different interpretations of central Arctic sediment cores; the (3) ice-cap theory is based on data more theoretical, and from outside of the Arctic Ocean.

Formation of the modern Arctic Ocean

Each of the three theories, presumably includes an evolution of the present ice-cover, from similar beginnings but through quite different stages of development.

(1) The 'fluctuating regime' theory[35–37] proposes that the late Miocene to Holocene interval was represented by three different palaeoclimatologic regimes, only the most recent of which (I), occurring from approximately 0.7 Myr to Recent, was characterized by a perennial ice-cover. Of the three theories, this is the most elaborate and involved. This theory suggests that during palaeoclimatologic regime III (interpreted to be roughly from 4 or 5 Myr ago to 2.7 Myr ago), the Arctic Ocean was cold but not ice-covered. The ocean had productive waters, interpreted by the theory's proponents to be the highest productivity of any time during the past 4.5 Myr. The productivity of the surface waters affected deeper waters, as well, because of good vertical mixing. The next younger or palaeoclimatologic regime II, is interpreted to be characterized by stratified saline and fresh water with little vertical mixing, resulting in both little surface productivity as well as no bottom productivity. Coccoliths were reported to occur during this interval[36] and ice-free conditions are suggested, at least for part of the year. This regime extended from 2.7 to 0.7 Myr ago. Finally, the most recent climate regime, I, is interpreted to have been initiated at about the same time as the Brunhes–Matuyama boundary (0.7 Myr), and at this time permanent ice, similar to that of the present, formed. This time interval is interpreted to be characterized by sharp and frequent fluctuations in foraminifera abundance, and sparse coccoliths. Sparsity of coccoliths is suggested to be due to intense bioturbation.

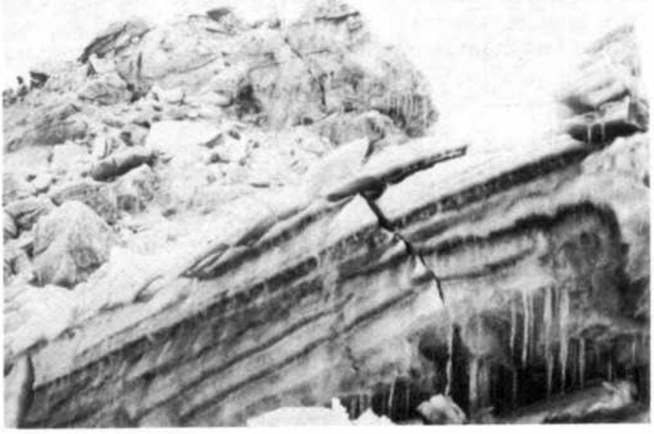

Fig. 3 Layers of fine silt and clay exposed in pack-ice that has been thrusted in a pressure ridge. Fine grained sediment is incorporated into newly forming ice during autumn storms in shallow shelf areas and then drifts seaward with surface currents. Pack-ice is a major source of fine grained sedimentation in the central Arctic Ocean.

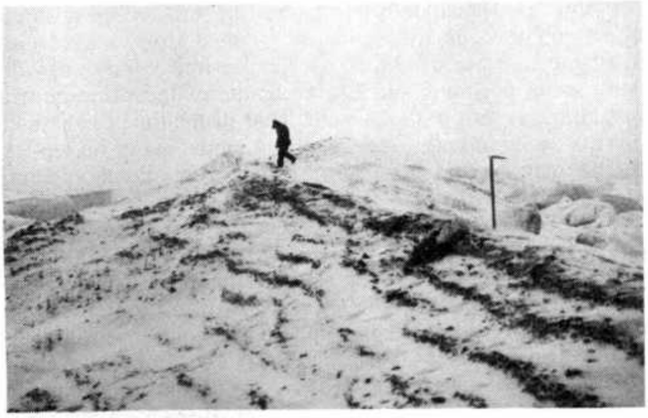

Fig. 4 Iceberg, moving with pack-ice of Arctic ice-cover, and showing glacial derived coarse grained sediment layers. Icebergs are the only source of coarse grained sedimentation in the central Arctic Ocean.

While, elaborate in design, many of the conditions interpreted to be necessary for this theory have a questionable basis. This may be due to the fact that fewer than a dozen dried-out and heavily sampled sediment cores from the limited Lamont collections were available for study. For example, strong vertical circulation and high productivity, interpreted for climate regime III, is based on abundance of Fe–Mn micronodules that are said to have foraminifera as nuclei. These micronodules, reported to characterize only regime III [35], actually occur in moderate abundance throughout the cores (sediment of regimes I and II, as well) and most do not have foraminifera as nuclei[7,19]. Regime II, said to have resulted from strongly stratified waters with little or not organic productivity, actually spans the time of the beginning of abundant foraminifera and, in general, the Arctic Ocean was more productive during this time than during time of regime III. O'Neill[19] studied 11 fresh sediment cores from along the flanks of the Alpha Cordillera and Canada Basin, some at 3–4 cm intervals. He recognized 75 benthic foraminifera species including 15 textulariid and miliolid species and 34 rotalid species, that range through the interval of regime II, said to be a time of nonproductive waters[35–37].

After comparing the fluctuating regime theory with the data of his cores, O'Neill concluded that the "high productivity model suggested by Herman and Hopkin[35], (for regime III) does not appear to be supported by the available data".

Inferences of red clay occurrence and bioturbation to depths of 30 cm during any of the regimes also cannot be verified[7,19]. Rather, glacial–marine sediment with erratics characterizes all sediment (from at least 5 Myr ago) and bioturbation cannot be interpreted to have affected more than 10 cm of sediment. Inconsistencies in the interpretation of coccolith data are also worrying. In July and August of 1980, Margolis and Herman[37] and Herman and Hopkins[35] reported that coccoliths occur in regime III sediment, but in October[36] no coccoliths nor discoasters were reported for regime III. In the final report[36], it turned out that 5 of the 6 samples said to have had nannofossils actually occurred in regime 1 sediment; the sixth sample (with two specimens) was from regime II. This was used as part of the evidence for ice-free condition. Ice-rafting of an occasional coccolith from marginal Arctic waters to the central Arctic may be a better explanation for all of the occurrences.

The fluctuating regime theory is difficult to verify because of inconsistencies in the reports as well as apparent misinterpretation of the sediment cores that form the basis for the theory.
(2) The 'steady state' theory[7,13,33,34] is also sediment based and argues that the same kind of glacial–marine sediment that accumulates today under an average of 3 m ice, has been accumulating for at least 5 Myr. Although there have been some changes in rate of sedimentation and in percentages of coarse material deposited, the changes have been of the same magnitude during most of the past 5 Myr and probably represent sedimentation in more or less similar conditions. The sediment record for several hundred cores has been organized into a lithostratigraphical sequence of 13 stratigraphical units[7]. The stratigraphical units consist of silty lutites reflecting lower sedimentation rates and corresponding to decreased ice-rafting, alternating with arenaceous lutites representing higher sedimentation rates and corresponding with increased time of ice-rafting. Clays and silts are transported both by pack-ice (frozen seawater) (Figs 2, 3) and glacial-ice (icebergs) but coarser sediment is only transported by icebergs[38] (Fig. 4). The lithostratigraphical sequence includes units that are fine-grained (probably principally rafted by pack-ice) and coarse-grained (rafted by glacial-ice). Because these units alternate throughout the 5 Myr for which the central Arctic record is more or less complete, both pack-ice and glacial-ice are considered to have been present since at least the Miocene. Older Cretaceous–Palaeocene material was recovered in two cores and their study was the basis for the interpretation that open water conditions existed during the late Mesozoic to early Cenozoic[5]. No sediment record has been recovered for the Eocene to late Miocene interval.

The steady state theory interprets the sediment record of the Arctic Ocean for the past 5 Myr to show little variation on a single theme, ice-rafting. Foraminifera and calcareous dinoflagellates show positive correlation during part of the Pleistocene (Fig. 5). Foraminifera abundance in the central Arctic Ocean and a percentage of coarse, glacial-ice-rafted material show inverse correlations throughout the interval when significant foraminifera have been present. Some effect of dilution by clastic influx is possible. Calcareous dinoflagellate occurrence also shows negative correlation with increased glacial ice-rafting[39] (Fig. 6). This is interpreted to indicate that during rapid ice decay and calving (inter-glacial intervals), surface waters may have been too low salinity to support foraminifera and dinoflagellates but that these organisms were relatively productive during stable glacial-growth (glacial intervals). The surface of the central Arctic Ocean could have been perennially ice-covered during the entire interval.

A fine tuning of this theory has resulted from the study of calcareous dinoflagellate fossils that show alternating times of abundance and rareness during the interval 2.0–0.7 Myr ago. Evidently, during this 1.3-Myr period, favourable conditions to encourage production of calcispheres occurred. Conditions before 2.0 Myr and after 0.7 Myr have been more like the present[39].
(3) The 'ice-cap' theory argues that Pleistocene sea level changes and oxygen isotope records from benthic foraminifera of the lower latitude oceans could be more easily explained if the Arctic Ocean had an Antarctic size ice-cap 1,000 m thick, during all or most of the Pleistocene[40–44]. This theory has evolved from ideas based on a West Antarctic analogy and explanations for displaced marine fossils in Alaska[40] and theoretical considerations suggesting that during the past 160,000 yr,

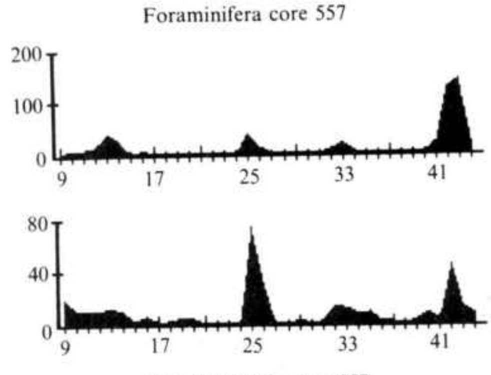

Fig. 5 Abundance of foraminifera and positive correlation with abundance of dinoflagellates for the interval 2.0–0.7 Myr ago in the central Arctic in core F1-557. Notice different vertical scales.

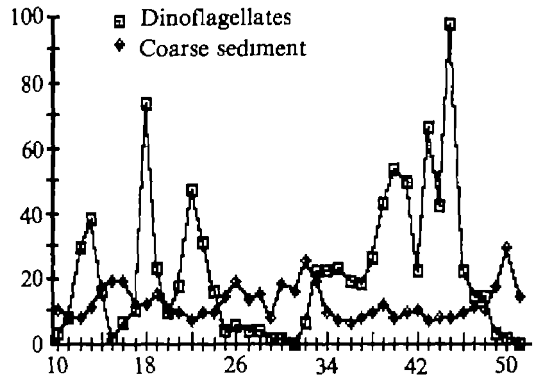

Fig. 6 Plot of abundance of dinoflagellates and percentage of coarse (glacially derived) sediment for core F1-301, central Arctic Ocean. General negative correlations seen in all cores studied, that is, time of occurrence of greatest percentage of coarse sediment (sample 50) correlates with time of lowest number of dinoflagellates and vice-versa (sample 45, 22 and so on).

the world benthic foraminifera ^{18}O record requires a larger ice volume than can be interpreted from sea level data[43,44]. The ice needed to explain the apparent discrepancy between sea level calculation and ^{18}O records of ice volume is interpreted to have been in the Arctic Ocean. A 50-m sea level difference, that would account for at least some of the discrepancy, would require a floating ice cap 1,300 m or more thick "close to the mean depth" of the Arctic Ocean[44]. Unfortunately, this theory ignores data from the Arctic Ocean. As pointed out earlier, during most of the 160,000 yr that a 1,000-m ice sheet is theorized to have been present, sedimentation similar to that of the modern Arctic ocean was in progress and various types of organisms lived, and at times, even flourished. It is difficult to understand phytoplankton and foraminifera thriving under >1,000 m of ice, as the ice cap theory demands. Clearly the evidence for relatively high organic productivity based on hundreds of short sediment cores that include the critical time interval, cannot be ignored[14,19]. Similarly, the mechanism that would permit accumulation of ice-rafted sediment beneath a >1,000 m ice-cap whose thickness is close to the mean depth of the ocean, is unknown.

This theory must accommodate the extensive data from Central Arctic Ocean sediment cores as well as theoretical considerations before it can be used to explain Pleistocene events.

Future CO_2-induced climate change and Arctic Ocean ice-cover

The three theories are concerned with the modern Arctic Ocean ice-cover and its geological history. Climatologists worried about the increasing atmospheric CO_2 are concerned about the melting of the Arctic ice cover[45-47] and future climatic effects. The early disappearance of the perennial Arctic ice-cover during a CO_2-induced climate change would produce a unipolar cryosphere, affecting sea surface temperature and producing a shift of climatic belts by several degrees latitude[3]. The climatic impact of an ice-free Arctic Ocean has received much consideration[48,49] although a sea level rise perhaps mostly related to Antarctic melting, may be in progress[50].

Flohn[3] has speculated that the best understanding of future effects on the climate could be obtained from a geological analogue; a time of Arctic Ocean ice-free conditions with continental positions and Earth climate of the same general magnitude as that of the present. That such a time did actually exist is disputed and it is argued that modelling of an ice-free Arctic may be the superior method of predicting climatic effects[51].

There is no widespread agreement that a realistic model of an ice-free Arctic Ocean has actually been produced, although there have been several interesting attempts[52-58]. Several of the models suggest that the ice could melt in the summer but even under considerable atmosphere CO_2 addition, winter ice would form, at least as long as the Earth's current orbital status persists. The climate change produced by an absence of summer ice and a reduced winter ice cover are matters for atmosphere modellers. No geological analogue with continents and climate patterns such as those of the present, has probably emerged for 5 Myr.

Conclusions

The role of the Arctic Ocean in Earth climate patterns has been significant, at least for the past 100 Myr. An open Arctic Ocean with extensive upwelling and free exchange with more southerly waters characterized the late Cretaceous–early Cenozoic. This is confirmed from central Arctic sediment[5,20,21] as well as from vertebrate and plant study in Arctic and sub-Arctic areas[59]. Formation of the Antarctic ice sheets along with development of glacial conditions in the Arctic were important factors for the general cooling of Earth during the Middle Cenozoic. Glacial–marine sediment was forming in the central Arctic at least 5 Myr ago. The first freezing of the Arctic surface waters may have been earlier than this but there is no direct sediment evidence to support this idea. This age probably is a minimum, as ice-rafted debris may have been accumulating in Svalbard as early as the Eocene[60].

From at least 5 Myr ago to the present, glacial–marine sediment, transported by ice-rafting of icebergs and pack-ice, has accumulated in the central Arctic Ocean. Generally, times of extensive ice calving are correlated with accumulation of coarser sediment and abundant erratics. These intervals of relatively rapid sedimentation (for the Arctic) alternated with times of accumulation of finer grained, predominantly ice-pack transported sediment and greater organic productivity. From ~2 to 0.7 Myr, calcareous dinoflagellates left a record of alternating abundances and rareness. Probably, during this time surface conditions permitted greater productivity than had been possible before 2 or since 0.7 Myr.

There is no evidence from the Arctic Ocean to support the idea of giant Ice Caps, 1,000 m thick, during any part of the Pleistocene. Evidence from modelling for future CO_2 induced climate changes predicts a reduced winter ice-cover and an absent summer ice-cover for the Arctic Ocean. If this occurs, significant climate changes are indicated. Unfortunately, no geological analogue is available. Rather, the only evidence from the central Arctic, suggests that conditions like those of the present, punctuated by times of greater iceberg activity, characterized the Arctic Ocean during most of the late Cenozoic.

Photographs are courtesy of Arnold Hanson, University of Washington.

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ARTICLES

A voltage-gated ion channel model inferred from the crystal structure of alamethicin at 1.5-Å resolution

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The crystal structure of alamethicin in nonaqueous solvent has been determined, and refined at 1.5-Å resolution. The molecular conformation of the three crystallographically independent molecules is largely α-helical with a bend in the helix axis at an internal proline residue. The helix structure is highly amphipathic as most of the solvent-accessible polar atoms lie on a narrow strip of surface parallel to the helix axis. Molecular models for the voltage-gated ion channel, with n*-fold symmetry and based on the molecular conformations observed in the crystal, are characterized by strong surface complementarity, a hydrophilic interior and a hydrophobic exterior. The channel structures are stabilized by a hydrated annulus of hydrogen-bonded glutamine residues which produce the greatest restriction in the channel diameter.*

THE linear peptide antibiotic alamethicin spontaneously inserts into black lipid membranes producing voltage-gated ion channels[1]. While alamethicin isolated from *Trichoderma viride* is a mixture of several related chemical species[2,3], the sequences of the major (and minor) components have been identified as[4,5]:

$$\text{Ac-Aib-Pro-Aib-Ala-}\overset{5}{\text{Aib}}\text{-}\overset{\text{(Aib)}}{\text{Ala}}\text{-Gln-Aib-Val-}\overset{10}{\text{Aib}}\text{-}$$

$$\text{Gly-Leu-Aib-Pro-}\overset{15}{\text{Val}}\text{-Aib-Aib-Glu-Gln-}\overset{20}{\text{Phl}}$$

although cyclic[2,6] and branched[7] structures have been proposed. The N-terminal residue of alamethicin is acetylated (Ac^-). The peptide contains eight or nine α-aminoisobutyric acid (Aib) residues and has L-phenylalaninol (Phl) as its C-terminal residue. The conductance properties of alamethicin in black lipid membranes have been extensively investigated and recently reviewed[8]. The macroscopic conductance is strongly voltage-dependent, increasing *e*-fold for every 4–6 mV in applied voltage, and also exhibits a strong alamethicin concentration and ionic strength dependence[1,9–12]. The high power dependence of the conductance on alamethicin concentration suggests that the alamethicin ion channel consists of 6–11 or more molecules. An ionic strength-dependent partitioning of alamethicin into a lipid phase appears to explain the observed dependence of conductance on salt concentration[10].

At low alamethicin concentrations, conductance fluctuations are observed which have been attributed to single-channel gating events[9,13,14]. Single-channel current bursts fluctuate

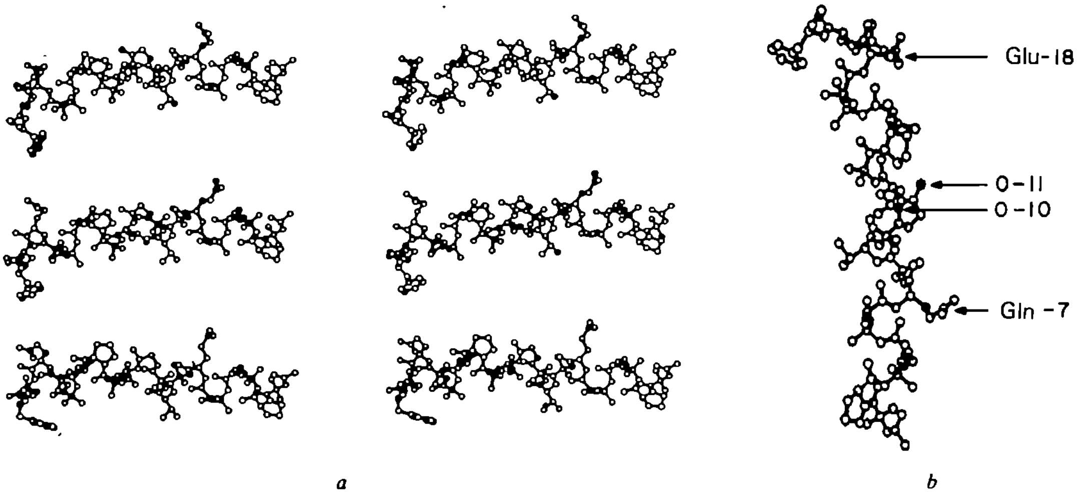

Fig. 1 *a*, The three alamethicin molecules present in the asymmetric unit are positioned in a similar orientation to the N-terminal segment. The N-terminus is to the right in each stereo diagram. The hydrophilic surface of each molecule projects towards the top of the figure. *b*, The solvent-accessible polar groups (see text) are labelled for molecule I. The hydrophilic surface of the molecule projects to the right.

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among several discrete conductance levels which are not integral multiples of each other. The most probable conductance substate and the lifetime of the current burst are only moderately voltage-dependent. The strong voltage dependence of the macroscopic conductance has been attributed to a change in the number of gated channels rather than a significant change in the properties of a single channel. The alamethicin channel is only weakly cation-selective[13,15-17]. The higher conductance substates are less sensitive to cation radius[16], indicating that the increase in current is due to a change in the cross-section of the channel, rather than an increase in the number of parallel open channels of similar area. Kinetic measurements of alamethicin-induced current are well modelled by a single exponential time constant, which is only moderately dependent on the alamethicin concentration in phosphatidylcholine membranes[11] and independent of peptide concentration in glycerol monooleate membranes[12]. These observations suggest that alamethicin exists predominantly in a preformed oligomeric state before a voltage is applied to the membrane.

The voltage-gating event must represent the movement of a charged group in the electric field or the orientation of a molecular dipole. The latter mechanism has been accepted in most models of alamethicin channel voltage-gating. The 'barrel stave' model of alamethicin action[14,18] suggests that peptide monomers at the surface of the alkyl chain region of the bilayer, are oriented by the electric field via coupling to the molecular dipole, and aggregate to form an oligomer once aligned with the electric field. The conductance substates are explained by a change in the channel cross-section due to a fluctuation in the number of monomer units in the oligomer. Hall[19] has proposed a two-dimensional micelle of alamethicin at the surface of the membrane which is oriented in the electric field via the molecular dipole, forming an oligomeric structure having a constant number of monomers, which fluctuates in an undefined way to generate the conductance substates.

The conformation of alamethicin is partially α-helical in organic solvents, as determined by circular dichroism[20,21]. Crystal structures of small peptide fragments of alamethicin and poly(α-aminoisobutyric acid) are in a 3_{10} helical conformation[22,23], although a larger Aib-containing peptide adopts an α-helical conformation in crystals[24].

We performed a crystallographic analysis of alamethicin to define the detailed molecular conformation and packing involved in the ion channel, and to differentiate among the proposed chemical structures[2,4-7]. This structure analysis has defined the helix type (α or 3_{10}) and the deviations from those ideal geometries, and illustrated the packing of Aib-containing helices. Knowledge of the molecular structure in the crystal has allowed model-building studies to define the proposed channel structure.

Structure determination and refinement

Alamethicin was provided by the Upjohn Company and was used without further purification[25]. Crystals suitable for diffraction were prepared by 10-fold dilution with acetonitrile of a solution of alamethicin in methanol (100 mg ml^{-1}). Integrated X-ray diffraction intensities were collected to 1.5 Å resolution by diffractometry using a modified Wyckoff scan[26]. An isomorphous heavy-atom derivative was prepared by diffusion of dimethyl mercury into the crystal from a mother liquor solution. Heavy-atom parameters and protein phase angles were refined[27] at 3.0 Å resolution. An atomic interpretation of the electron density map was constructed and refined using the distance-restrained least-squares procedure of Konnert and Hendrickson[28]. Calculated phases from the refined model were used to extend the resolution from 3.0 Å to 1.5 Å.

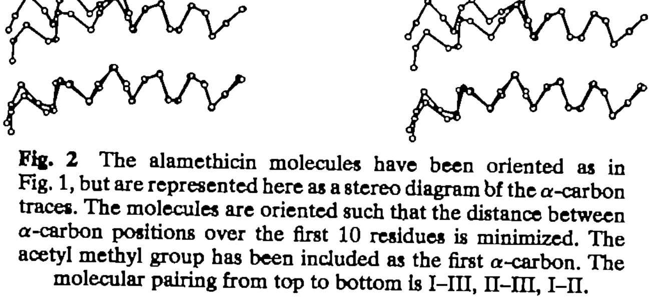

Fig. 2 The alamethicin molecules have been oriented as in Fig. 1, but are represented here as a stereo diagram of the α-carbon traces. The molecules are oriented such that the distance between α-carbon positions over the first 10 residues is minimized. The acetyl methyl group has been included as the first α-carbon. The molecular pairing from top to bottom is I–III, II–III, I–II.

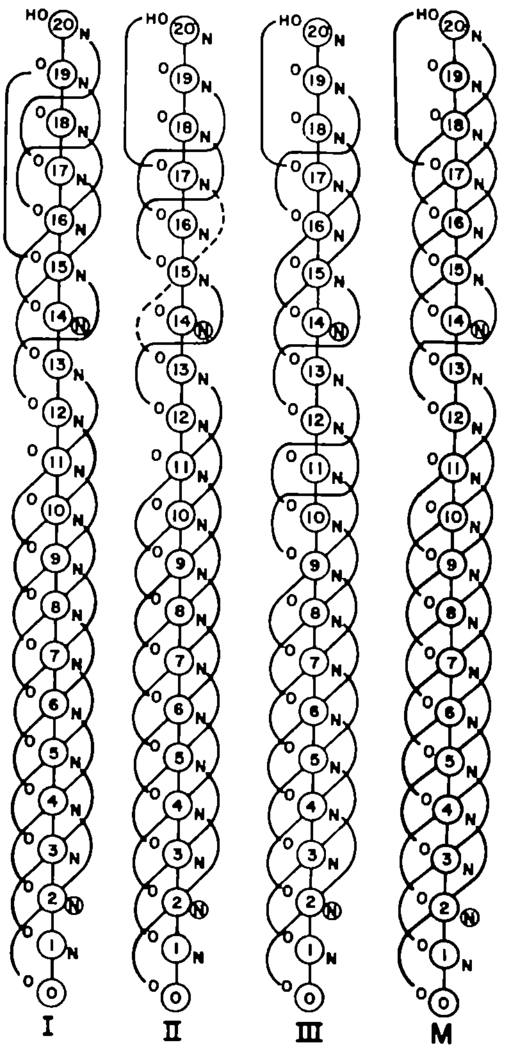

Fig. 3 The intramolecular hydrogen-bonding pattern for each of the three independent alamethicin molecules (I, II, III) is indicated together with the connections used in the channel model building (M) as described in the text. Residue 0 represents the acetyl group; circled N symbols, proline residues. The broken lines indicate an unusually long interaction. Hydrogen bonds of the 5→1 type (α-helix) cross the vertical diagonally while 4→1 hydrogen bonds (3_{10} helix) are represented by lines crossing the vertical axis horizontally.

Molecular models of the proposed channel structures were constructed with Corey–Pauling–Koltun (CPK) units and by a molecular graphics program using an Evans and Sutherland PSII display system. Space-filling drawings were generated on a colour raster graphics unit (Advanced Electronics Design, Inc.) using the program of Connolly[29].

Crystals of alamethicin are of space group $P2_1$, $a = 33.33$, $b = 29.62$, $c = 23.20$ Å, $\beta = 120.4°$ and contain three molecules per asymmetric unit. The solvent content of the crystals is 30%. The present analysis was carried out at 1.5 Å resolution although diffraction maxima have been observed to 1.0 Å. The distance-restrained least-square refinement converged with a crystallographic residual of 15.5% and a r.m.s. deviation from ideal bond lengths of 0.04 Å. Details of the crystal structure determination and refinement will be presented elsewhere.

Molecular structure and crystal packing

The crystal structure of alamethicin is consistent with the linear backbone structure[4,5], and excludes the cyclic[2,6] and branched[7] structures from further consideration. The side chain density of the final $(2F_0-F_c)$, model-phased, electron density map, calculated from the complete molecular model, reveals no deviation from the chemical sequence proposed for the major component of alamethicin[5]. It is unlikely that side chain heterogeneity due to minor components, if they were in fact present in the crystals, would be observed in the electron density map described above.

In general, all three alamethicin molecules in the asymmetric unit exist in an α-helical conformation over their entire lengths. There are, however, small deviations from a continuous α-helical conformation in the form of short (one to two residues) 3_{10} helical regions in the C-terminal domain. Figure 1 shows a stereo diagram of the three independent molecules. Each helix axis is bent slightly and to a different extent near the Pro-14 residues. The angular variability of the bend and the variations in helix parameters seem to be due to the differences in intermolecular polar interactions among the three independent alamethicin molecules. Figure 2 illustrates the difference in bend angles among the three molecules (I, II, III), and Fig. 3 gives the intramolecular hydrogen-bonding pattern for each molecule.

The accommodation of the Pro 14 residue into the α-helical backbone is accomplished by the introduction of a 4 → 1 hydrogen bond (N-15 to O-12) which shifts the proline imino ring from a position directly above the carbonyl of Aib 10 to one between the carbonyls of Aib 10 and Gly 11. The Pro 14 imino ring is further accommodated by the bend in the helix axis away from the Pro 14 ring direction. In Fig. 2 the molecules have been superposed such that the overlap of like α-carbons for the first 10 residues is maximized between each pair of molecules. Molecules I and II display a similar bend angle, deviating significantly only at the C-terminal residues where the hydrogen-bonding pattern differs. Molecule III displays a much smaller bend in the helix at the Pro 14 residue, which is accommodated in part by a displacement of the Gly 11 carbonyl away from the helix axis.

The solvent accessibility[30] was calculated for each isolated alamethicin molecule without neighbouring peptide or solvent molecules. The solvent-accessible surface area is generally less than 5 Å for carbonyl oxygen atoms participating in backbone hydrogen bonds. The carbonyls of Aib 10 and Gly 11 do not participate in backbone hydrogen bonds and have solvent accessibilities between 5 Å^2 and 17 Å^2. The carbonyl oxygen atoms fully exposed at the C-terminus of the molecules have calculated areas of 18–30 Å^2. The carbonyl groups of Aib 10 and Gly 11 of molecule I are hydrogen-bonded to methanol solvent molecules, while the carbonyl of Gly 11 of molecule III is hydrogen-bonded to the amide side chain of Gln 7 from molecule II. The carbonyls of residues 10 and 11 of molecule II and 10 of molecule III do not form hydrogen bonds with the solvent structure as it is presently modelled.

The alamethicin sequence contains four residues having polar side chain groups: Gln 7, Glu 18, Gln 19 and Phl 20. The Phl 20 hydroxyl group hydrogen bonds to the backbone in two of the three independent molecules. The carbonyl oxygen atoms of Aib 10 and Gly 11 are solvent-accessible and must also be considered as polar regions on the alamethicin molecular surface. Although the backbone structure differs among the three independent molecules in the C-terminal region, the distribution of polar groups on the surface of each molecule is very similar. The Gln 7 and Glu 18 side chains and the carbonyl oxygen atoms of Aib 10 and Gly 11 all lie on a narrow strip on the surface of each molecule parallel to the helix axis. The polar strip lies on the convex surface of the bent helices. The

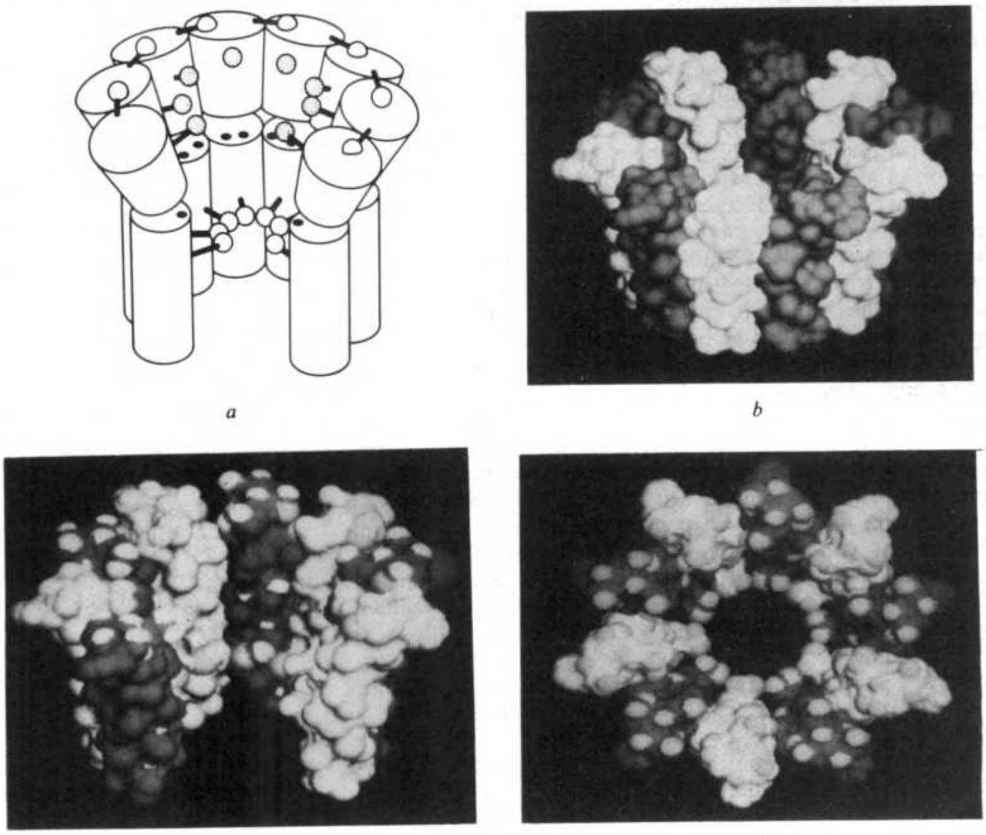

Fig. 4 *a*, Schematic and computer graphics representations of the proposed pore. The alamethicin monomer as seen in the crystal is shown as two cylindrical segments signifying the interruption of the α-helical hydrogen bonding introduced by the Pro 14 residue. The stippled spheres at the mouth of the channel represent the Glu 18 residues while the open spheres at the centre and top of the channel structure indicate the hydrogen-bonded annulus of Gln 7 and Gln 19 residues, respectively. The parallel cylinders represent the N-terminal domain of the alamethicin molecules. The black dots on the face of each cylinder represent the solvent-accessible carbonyl groups of Aib 10 and Gly 11. The upper cork-shaped elements represent the C-terminal domains, and are tipped away from the channel *n*-fold axis, illustrating the bend imposed by the Pro 14 residue. The C-terminal elements are flaired to indicate the increasing bulk of the side chains in this region, maintaining van der Waals' contact even as the helix axes diverge. The stippled spheres represent the Glu 18 side chains, which are removed from direct contact due to the flair of the C-terminal region. The Gln 19 side chains are represented by the spheres at the top of the C-terminal segments illustrating the hydrogen bond interactions with the carbonyl oxygen atoms of a neighbouring molecule. *b*, A solvent-accessible surface representation of the complete alamethicin channel exterior, illustrating the strong surface complementarity of the hydrophobic exterior surface of the N-terminal region. *c*, A cutaway view of the alamethicin channel as in *a*, representing the solvent-accessible surface of each molecule. Alternate molecules are shaded light and dark to illustrate the intermolecular packing: the lighter shading represents solvent-accessible oxygen and nigrogen atoms of the backbone and side chains. Molecule I was used to form the channel structure without modification, thus modifications to the C-terminal regions of the molecule which would allow Gln 19 hydrogen bonding to neighbouring molecules have not been made. Also, the Gln 7 annulus does not seem as restricted as was observed in the CPK modelling, as the side chain geometry has not been altered to agree with the physical model. *d*, A view down the oligomer axis from the C-terminal surface.

Gln 19 side chain does not lie on this polar strip. Thus, the alamethicin structure is amphiphilic in two respects, as most of the polar groups are segregated in the C-terminal region of the primary structure, and lie along a narrow polar strip parallel to the helix axis in the tertiary structure.

The orientation of the three independent alamethicin molecules does not result in a symmetric trimer structure, but the independent molecules pack with each other and with molecules related by 2_1-symmetry operations and translations to form a complex array of irregular channels, parallel to the x-axis of the unit cell. Although the alamethicin molecules pack to form solvent-filled channels, the polar residues do not segregate towards the centre of the channel as might be expected for a membrane ion channel. In any single solvent channel, both faces of a single molecule type contribute to the channel surface. The three independent molecules, identified as the asymmetric unit, are oriented in parallel with their C-termini close to the origin and their N-termini directed in the $+x$ direction, perpendicular to the 2_1-symmetry axis. Molecules of the asymmetric unit pack in an antiparallel arrangement with symmetry-related molecules.

Construction of alamethicin channel models

For the construction of models of the alamethicin pore, a 'mean' monomer unit was used, representing essentially an average of the three peptide conformations observed in the crystal structure. The alamethicin conformation was idealized as two regular α-helical segments (1–13 and 14–20) with an angle of 20° between the two helix axes. A single $4\rightarrow1$ hydrogen bond (Val 15 to Leu 12) was introduced as seen in each of the monomers in the crystal. Such monomer units were assembled into oligomers using the following rules: (1) n-fold symmetry around the membrane plane normal, (2) asymmetric distribution between the two sides of the membrane, (3) optimization of side-chain packing between monomer units, (4) maximization of intermolecular hydrogen bonding. Two alamethicin CPK models were docked by hand in various orientations and the fit was assessed subjectively. One set of orientations resulted in a particularly favourable packing of side chains in the N-terminal domain; in this orientation the helix axis of the N-terminal segments is parallel to the oligomer axis. The relative rotation about the helix axis is such that the Gln 7 amide groups from neighbouring molecules form hydrogen bonds with good geometry. When such interactions are propagated, complete channels containing 8–12 molecules are generated. The most favourable oligomer size cannot be determined from these model-building studies, however the hydration structure described below favours an octameric channel structure.

The side chains of the N-terminal domains interdigitate to form a zipper-like structure. All side chains participating in this interface are short (Ala or Aib), consisting of only β-carbon atoms. The Pro 14 residue is directed towards the centre of the channel, causing the helix axis of each molecule to bend away from the oligomer symmetry axis (Fig. 4). The molecules still maintain van der Waals' contact in the C-terminal domain interface but with less favourable surface complementarity, as the side chains in this region are bulkier. The channel model has a funnel-like appearance with a flair at the C-terminal domain region, due to the bend in the helix axis induced by the Pro 14 side chain.

The interior of the channel contains three bands of polar groups capable of hydrogen bonding to water. Each Gln 7 residue donates and receives a Gln 7 hydrogen bond involving residues of each neighbouring molecule, forming a hydrogen-bonded annulus within the channel (Fig. 4). The amide plane of each Gln 7 side chain is tipped out of the mean plane of the Gln 7 residues; each amide is tipped in a direction opposite to that of its neighbours, resulting in a zig-zag pattern. The resulting structure projects amide hydrogen bond donors from each Gln 7 side chain towards the oligomer symmetry axis such that the amide protons are alternately directed above and below the mean Gln 7 plane.

The Gln 7 side chains are in a relatively low energy conformation while making two hydrogen bonds with good geometry. Such considerations indicate that the hydrogen-bonded annular structure will be relatively stable even in the presence of channel water. The unhydrated Gln 7 annulus produces a 7 Å diameter constriction in the octameric channel.

The carbonyl groups of Aib 10 and Gly 11 are solvent-accessible in the model as found for isolated molecules in the crystal. Such hydrogen bond acceptors will provide a further means of solvating the channel interior.

The Glu 18 side chains are also directed towards the oligomer symmetry axis, but because the helix axes of the monomers are directed away from the oligomer axis in this region of the molecule, they do not make direct contact. Indeed, the oxygen atoms of neighbouring carboxyl groups are separated by a minimum distance of ~3.6 Å. The Glu 18 carboxyl groups can be further separated if strict n-fold symmetry is not obeyed. In an aqueous salt solution at neutral pH, the Glu 18 side chain carboxyl groups would be ionized but would be sufficiently shielded from each other by a cloud of condensed counterions so that they would not greatly destabilize the channel structure.

The Gln 19 side chain is tangential to the C-terminal rim of the oligomer, such that the carbonyl is directed towards the oligomer axis while the two amide protons hydrogen bond to oxygen atoms of a neighbouring molecule. The hydrogen bonds are made to the hydroxyl of Phl 20 (which in turn is hydrogen-bonded to the carbonyl oxygen of Aib 17 and to the carbonyl oxygen of Glu 18). This hydrogen-bonding system continues throughout the oligomer structure, stabilizing the C-terminal domains and in part counteracting the residual electrostatic repulsion of the Glu 18 residues. The stabilization realized from the Gln 19 interactions will depend on the hydrophobicity of that region of the membrane.

The exterior of the ion channel is highly hydrophobic, containing many small alkyl side chains and no polar side chains.

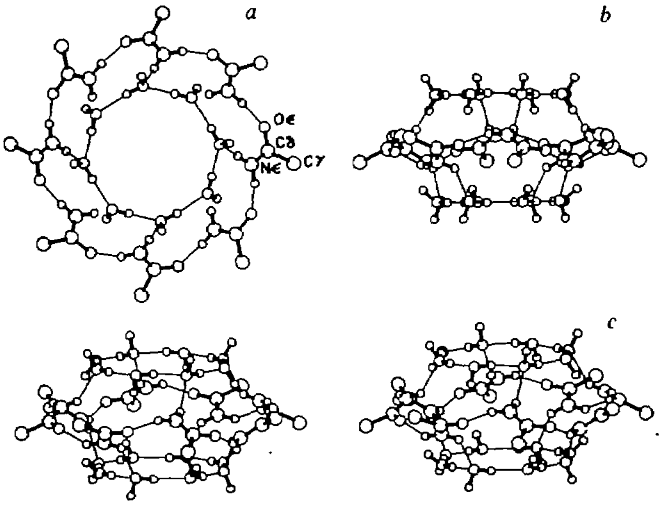

Fig. 5 Ball-and-stick drawings of one possible structure of the solvent near the Gln 7 annulus. *a*, The hydrated Gln 7 annulus viewed along the axis of the octomeric channel model. The atoms of one side chain are labelled. Only atoms beyond C^β are shown. The outer ring hydrogen bonds connect amide and carbonyl groups in adjacent Gln residues; the inner ring of hydrogen bonds connects eight water molecules to each other. The roughly radial bonds connect the unused proton of alternating amide groups to four of the water oxygen atoms. For clarity, the lower ring and interstitial water molecules are not shown. *b*, The hydration structure of the Gln 7 annulus perpendicular to the oligomer axis. The alternating orientation of the Gln 7 side chains can be seen together with the hydrogen bonding to both rings of water. *c*, A stereo diagram of the hydrated Gln 7 annulus. Four additional water molecules can easily be added inside the cage with many hydrogen-bonding possibilities. The mass density of such a 14-molecule hydration complex would be close to that of bulk water.

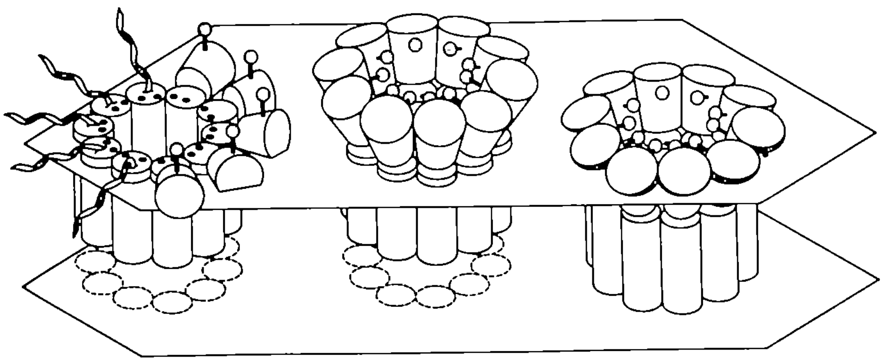

Fig. 6 Voltage-gating channel model. The parallel planes specify the boundary of the alkyl chain region of a lipid bilayer. Elements of the channel model which are in common with Fig. 4*a* are as described in legend. The left-hand channel illustrates the structures proposed for the alamethicin oligomer in the absence of an applied voltage. The helical ribbons signify an extended random coil structure. The cork-shaped elements represent the C-terminal region of each molecule, bent away from the channel *n*-fold axis and partially buried in the alkyl chain region. The channel model in the centre represents a hypothetical intermediate produced by an applied voltage. The model on the right is proposed as the open form of the ion channel.

The conformation of the model used in the construction of the oligomer contains only one non-hydrogen-bonded amide within the central region of the α-helix; this amide is located on the exterior of the channel.

The length of the channel along the oligomer axis is 32 Å, which is about the thickness of the alkyl chain region of biological and black lipid membranes. Thus, each end of the alamethicin molecule will be at the interface of the head group and alkyl chain regions of the lipid bilayer.

The distribution of polar groups in the alamethicin channel is much more regular than that found in globular proteins, as the polar groups within the channel occur in bands at different elevations. The first hydration layer within the channel must interact with this regular array of polar groups in a limited number of orientations, leading to a hydration structure which may be much more regular and extensive than that normally found surrounding globular proteins in crystals or in solution.

Ordered water structure may be of functional significance in the region occupied by the Gln 7 residues. The Gln 7 annulus is the most restricted region of the channel and contains a regular array of hydrogen bond donors directed towards the oligomer axis. The details of the hydration structure will depend on the number of monomers in the alamethicin oligomer. In general, each Gln 7 side chain amide proton will donate a hydrogen bond to a water oxygen atom, causing a further constriction in the ion channel. Such hydrogen-bonded water molecules would be further stabilized by a second set of water molecules bridging the first hydration layer, resulting in a hydrogen-bonded ring of water above and below the Gln-7 annulus. The hydrated Gln 7 annulus for an octameric channel is illustrated in Fig. 5. The diameter of the hydrated annulus region accessible to bulk solvent molecules or ions is ~4.5 Å for the octamer.

Functional implications of the channel model

We suggest that changes in the size or geometry of the Gln-7 annulus are responsible for the conductance substates observed in single alamethicin channels, as this structure provides the greatest restriction to the alamethicin channel cross-section. The model-building studies have not led to a unique channel structure, but to a family of oligomer models containing 8–12 monomers. Thus, the barrel-stave model[14,18] cannot be excluded by model-building studies alone. A channel oligomer of fixed composition could give rise to the conductance substate fluctuations if the stability of the Gln 7 annulus is altered by the applied voltage. The dipole moment of each Gln 7 side chain amide group is oriented almost perpendicular to the electric field in the channel model presented above. If the electric field is sufficient, the amide group will reorient such that the carbonyl is directed towards the C-terminus of the channel. Such a reorientation would result in an enlargement of the channel diameter while exposing a Gln 7 carbonyl group which could interact favourably with a hydrated cation, thus providing a path for cation movement through the channel. Such a reorientation would disrupt the hydration structure, which would also lead to an increase in the channel cross-section. The involvement of different numbers of amide groups in such reorientation might explain the fluctuating conductance substates, although persistence for milliseconds implies the existence of activation barrieo iation whose structural origin is not yet clear.

A molecular explanation of the voltage-gating event requires an understanding of the alamethicin molecular structure in the resting membrane and the physical basis for the voltage-driven structural change. A physical model for the voltage-gating process is presented in Fig. 6. We suggest that the hydrophobic N-terminal segment of alamethicin (residues 1–10) inserts into the alkyl chain region of the lipid bilayer without the aid of an applied voltage, forming a solvent-filled channel traversing one-half of the alkyl chain region. This preferential insertion is expected because of the small number of solvent-accessible polar backbone and side chain atoms present in that segment relative to the more hydrophilic C-terminal region. The insertion of the N-terminus of the alamethicin α-helix into the lipid bilayer results in a favourable orientation of the molecular dipole moment in the electric field generated when that side of the membrane is at a positive potential. The C-terminal portion of the molecule is modelled either as a random coil, associating with the head group region of the bilayer or projecting into the solvent, or as an α-helical segment bent away from the channel *n*-fold axis. The lack of hydrogen bonds to the carbonyl groups of residues 10 and 11 (Fig. 3) which project towards the channel *n*-fold axis, would allow such a hinge motion without disrupting any hydrogen bonds observed in the crystal conformers or as proposed in the model, and would allow the hydrophobic exterior surface of the C-terminal segment to partition into the alkyl chain region of the bilayer. The dipole moment of the C-terminal helical segment would be perpendicular to the electric field direction in the bent helix model, while the dipole moments of the individual peptide groups would be randomly oriented if the C-terminal segment were in a random coil conformation.

An electric field applied to the alamethicin molecules in the sense used in conductance experiments would favour either a coil–helix transition or a reorientation of the C-terminal helical segment, both resulting in the intermediate shown in the centre of Fig. 6. This C-terminal exterior surface now presents a larger hydrophobic surface to the head group and aqueous phase region of the membrane. The burial of this surface in the alkyl chain region of the bilayer would thus serve as the driving force for the voltage-gating event. The inserted alamethicin channel is of sufficient length to span the alkyl chain region of the bilayer but would not extend significantly into the head group region.

Recently, two results have been published which are consistent with the proposed structure of the ungated alamethicin oligomer illustrated in Fig. 6: (1) cross-linking of a lipid photolabel to alamethicin in liposomes results in labelling of only the N-terminal segment (residues 1–13), indicating that this portion of the peptide is accessible to the alkyl chain region of the bilayer[31]; and (2) electron micrographs of negatively-stained liposomes containing alamethicin reveal a circular stain-excluding structure which is stained at its centre[32].

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The sequences of an expressed rat α-tubulin gene and a pseudogene with an inserted repetitive element

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The rat genome contains two segments closely related to a rat α-tubulin mRNA. Both have been cloned and complete nucleotide sequences are presented. Analysis of the structure and sequence of one of these establishes it as a functional α-tubulin gene. The second segment is a processed α-tubulin pseudogene. Comparison of this pseudogene to the mRNA and gene coding for α-tubulin strongly suggests that a mature mRNA was involved in its origin. Features of the pseudogene and a dispersed repetitive element inserted within it possibly reflect a common RNA-mediated process of insertion.

MICROTUBULES are ubiquitous cellular structures found in eukaryotic organisms and responsible for a variety of functions. The basic subunit of the microtubule is a heterodimer composed of two proteins, the α- and β-tubulins. The amino acid sequence of each of these proteins is conserved and their gene sequences cross-hybridize among distantly related organisms[1]. The multiple functions of microtubules might reflect multiple tubulin proteins with different amino acid sequences and thus multiple genes. Amino acid sequence[2] and nucleotide sequence data[3,4] have shown that mammalian α-tubulin protein is microheterogeneous within species. Whether this microheterogeneity reflects functionally specific α-tubulin proteins is not known.

All tubulin genes are probably derived from a common ancestral sequence. Analysis of the number of α- and β-tubulin genes in mammals using cDNA sequences as probes has detected 10–20 copies of each per genome[5–7]. These segments could either represent functional genes or nonfunctional pseudogenes. On the basis of their sequence organization, pseudogenes can be divided into two classes: simple duplications of the expressed loci with subsequent divergence of nucleotide sequences, or copying of sequences present in RNAs and integration of these cDNA-like segments. The latter type has been referred to as processed pseudogenes[8].

It has been proposed that pseudogenes of small nuclear RNAs were created through RNA intermediates[9]. These pseudogenes are typically flanked by short direct repeats ,that are thought to have been generated during integration of the pseudogenes. Many dispersed repetitive elements in mammalian DNA, such as human Alu elements, are also flanked by short direct repeats, perhaps also created by their insertion (reviewed in ref. 10).

The similarity in structure among processed pseudogenes, pseudogenes of small nuclear RNA and Alu dispersed repetitive elements suggests that these all could result from a common process involving RNA. We report the sequence of an expressed rat α-tubulin gene, a processed pseudogene derived from it and a rat dispersed repetitive element inserted into this pseudogene. The structures of these elements are consistent with the hypothesis that the pseudogene and the dispersed repetitive element were created through RNA intermediates.

Cloning of rat DNA segments closely related to rat α-tubulin mRNA

The sequence of a cDNA clone of rat brain α-tubulin mRNA has been described previously[4]. The cDNA clone pILαT1 is 1,617 nucleotides in length and possesses sequences from the 3′ poly(A) tract, through the coding sequences to within approximately 33 nucleotides of the 5′ terminus of an abundant α-tubulin mRNA. Twenty-nine of these 33 nucleotides were determined by sequencing primer extension products[4]; the total sequence is shown in Fig. 3.

Others have detected multiple copies of genomic α-tubulin sequences in a variety of species[11–14]. To determine the number of α-tubulin genes in rat, three probes were prepared from the α-tubulin cDNA clone; probes I and II encompass sequences of the amino and carboxyl terminal portion of α-tubulin, respectively, while probe III contains sequences totally included within the 3′-untranslated portion of the mRNA. As α-tubulin is a highly conserved protein, probes I and II should hybridize to most of the α-tubulin related sequences in the rat genome.

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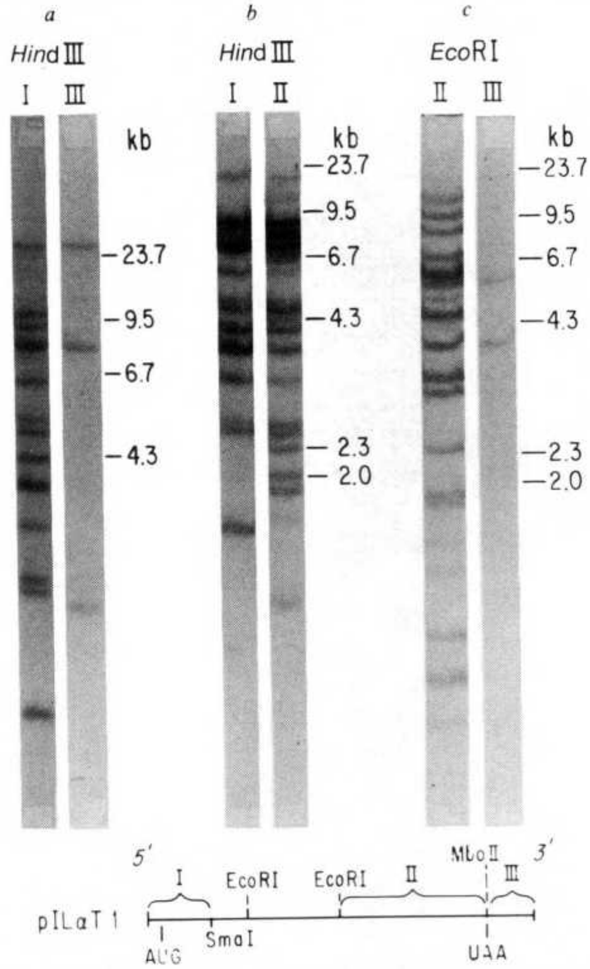


Fig. 1 Genomic representation of different portions of α-tubulin cDNA. Sprague–Dawley rat liver genomic DNA was extracted[37] and cleaved to completion by either *Hin*dIII or *Eco*RI endonucleases, as indicated above each set of lanes. *Hin*dIII does not cleave the cDNA pILαT1, while *Eco*RI cleaves the cDNA at two positions. The spectrum of fragments was resolved by electrophoresis (10 μg per lane) and transferred to nitrocellulose[38]. cDNA probes were prepared by digestion of pILαT1[4] with the indicated restriction endonucleases and isolation of appropriate fragments from preparative agarose gels by the sodium iodide-glass bead method[39]. ^{32}P-labelling was performed by nick-translation[40]. Hybridization and washing (see below) of blots was done essentially as described previously[4]. Three different probes were used: probe I encompassing 66 nucleotides of the 5′ untranslated (UT) and 190 nucleotides of the amino-terminus portion of α-tubulin mRNA, probe II consisting of 590 nucleotides of COOH-terminal information and ending 5 nucleotides before the termination codon and probe III, totally contained in the 3′-UT portion of the mRNA. *a*, probe I hybridizes to 16 fragments. All of the 16 bands persist through stringent washes (0.5–2 × SSC, 65 °C). Hybridization of genomic blots with probe II (*b* and *c*) allows an estimate of 14 copies of sequences homologous to the carboxyl-terminal portion of α-tubulin mRNA. In total, 10 fragments hybridize to both probes I and II (part B). The lane in *a* probed with III and washed at a moderate stringency (2 × SSC, 65 °C) contains four different fragments homologous to the 3′-UT portion of α-tubulin mRNA. However, in *c* only two fragments in an *Eco*RI digest remain hybridized after a high stringency wash (0.2 × SSC, 65 °C). Therefore, the rat genome contains only two copies of α-tubulin 3′-untranslated sequences closely related to the mRNA represented in pILαT1.

Sequences in the 3′-untranslated region of the mRNA should be less constrained and could perhaps be used to distinguish between rat genomic sequences closely related to the cDNA clone and those more distantly related. This is in fact the case. The amino-terminal and carboxyl-terminal probes hybridize to 16 and 14 *Hin*dIII fragments of rat DNA, respectively (see Fig. 1*a*, *b*). Ten of these bands hybridize to both probes, suggesting that these fragments contain the entire α-tubulin coding sequence. However, only two *Eco*RI bands hybridize in stringent conditions to the probe from the 3′-untranslated portion of the mRNA, suggesting that there are only two genes closely related to the cDNA clone (see Fig. 1*c*).

λ-Phage libraries of rat liver DNA sequences were screened by the method of Benton and Davis[15] with radioactive probes from both the 5′- and 3′-untranslated (UT) portion of the cDNA clone pILαT1. We had shown previously that these two probes hybridize in stringent conditions to the same set of genomic bands in a Southern blot. Seven phage plaques detected as positive with both probes in the first screen yielded positive hybridization signals in subsequent screening and these independent phages were selected for further study. Southern blotting of restriction endonuclease digests of the phage DNAs distinguished two phages, λαT15 and λαT14, that hybridized to the probes under high stringency. These two phages clearly had different restriction cleavage patterns.

Mapping of α-tubulin sequences within recombinant phages

Electron microscopy was used to map sequences complementary to α-tubulin mRNA in both λαT15 and λαT14. First, hybridization and R-loop mapping showed that both phage

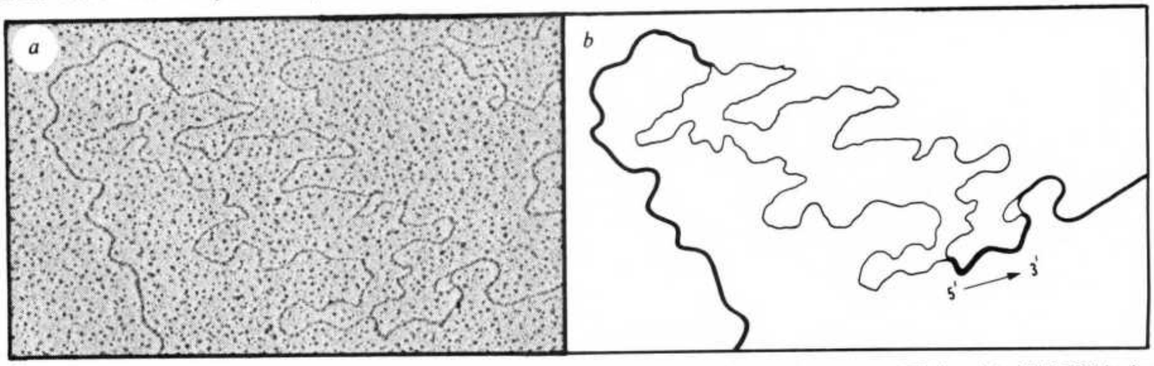
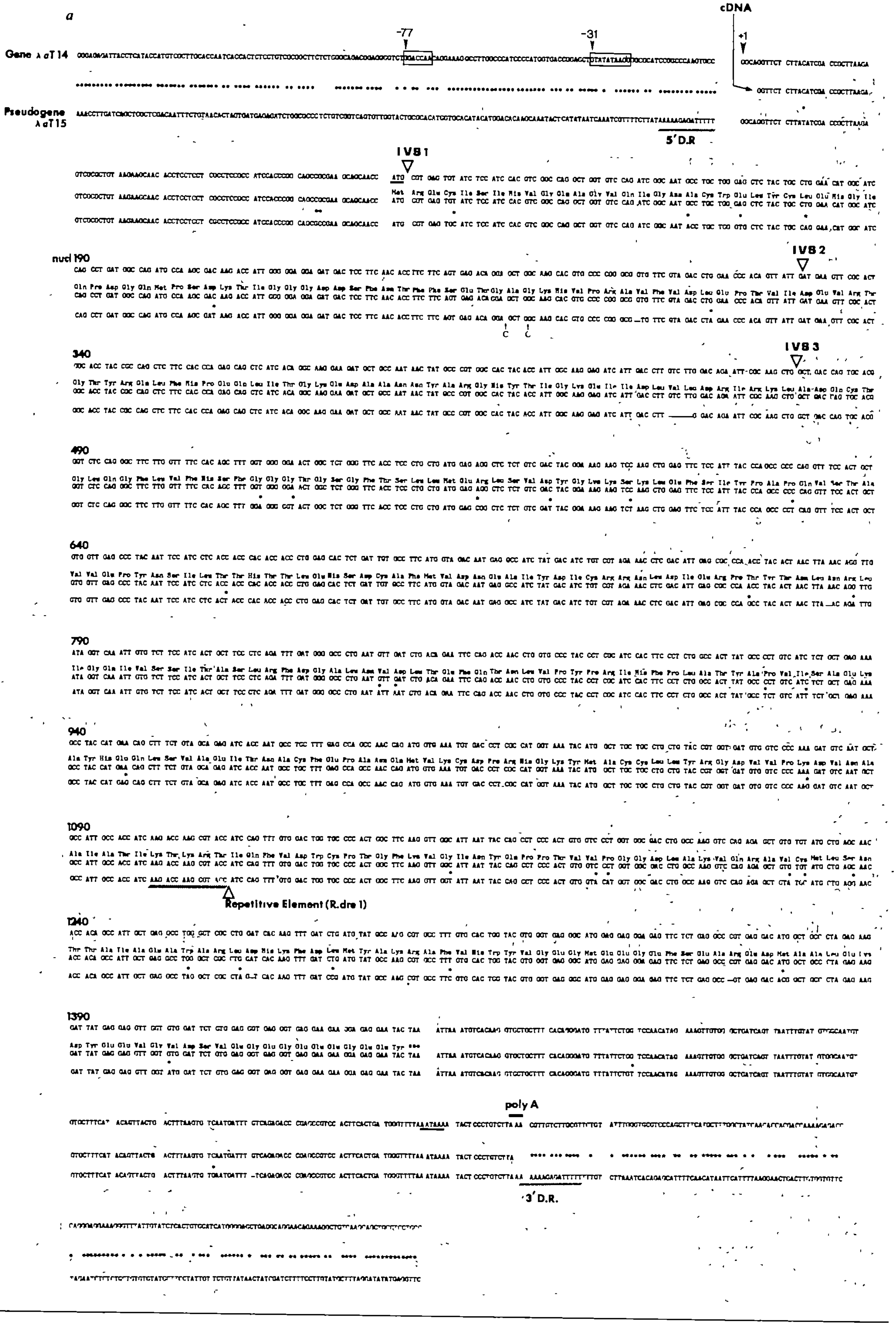


Fig. 2 Electron microscopic analysis of recombinant phage DNA. A heteroduplex formed between λαT14 and λαT15 DNAs is shown in *a* and represented diagrammatically in *b*. Hybrids were formed and mounted as described[41] with minor modifications. A several-hundred-nucleotide region of non-homology separates a 1,590 ± 110-nucleotide duplex segment from the long arm of the Charon 4A vector DNA. The position of this double-stranded region with respect to the positions of R-loops (formed by hybridization of mRNA and phage DNA; not shown) and with respect to restriction and blotting data established this as the segment homologous to α-tubulin cDNA. This combination of data also allowed the assignment of the designated polarity with respect to α-tubulin mRNA sequences.


a
cDNA
-77
-31
+1
Gene λαT14
Pseudogene λαT15
5'D.R
IVS1
nucl 190
IVS2
340
IVS3
490
640
790
940
1090
Repetitive Element (R.dre 1)
1240
1390
poly A
3'D.R.

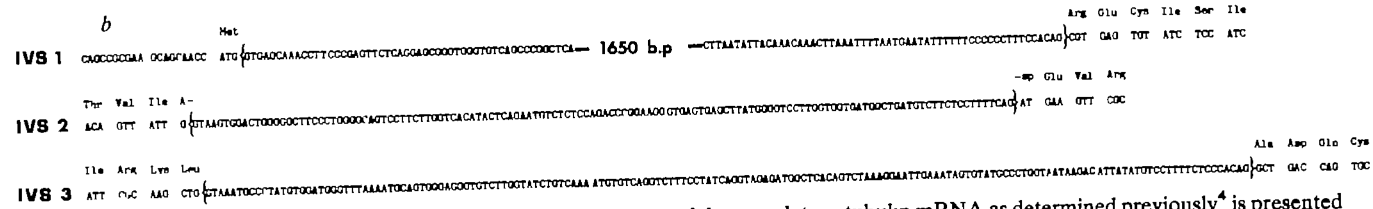


Fig. 3 Sequence of α-tubulin gene and pseudogene. *a*, The sequence of the complete α-tubulin mRNA as determined previously[4] is presented along with the sequence of the α-tubulin gene ($\lambda\alpha$T14) in the correct alignment above the mRNA. Sequences upstream of the mRNA initiation nucleotide (+1) are numbered beginning at −1. Putative transcriptional regulatory regions, GTATATAAGG at −31 and GGACCAA at −77 are boxed. The ATG initiation codon is underlined. Positions of intervening sequences are denoted (IVS1–3). The α-tubulin mRNA is polyadenylated at one of four terminal residues (overlined and labelled poly A). Note that the coding sequence of the gene is identical to that of the mRNA. The sequence of the α-tubulin processed pseudogene ($\lambda\alpha$T15) is aligned below the sequence of the mRNA. Comparison with the gene reveals close homology up to the mRNA initiation nucleotide (+1) and no significant homology further upstream (mismatches are indicated by asterisks). Several small deletions and two single base insertions into the α-tubulin mRNA homologous sequences in $\lambda\alpha$T15 are represented by underlined gaps or arrows, respectively. All three intervening sequences are precisely absent. The $\lambda\alpha$T15 sequences homologous to α-tubulin mRNA are immediately flanked by short 14-nucleotide direct repeats of AAAAAGAGATTTTT (underlined and labelled 5′ or 3′ D.R.). Note that comparison of 3′-flanking sequences in $\lambda\alpha$T14 and $\lambda\alpha$T15 reveals no significant homology. The position of the inserted repetitive element (R.dre.1) found in the pseudogene is shown by an arrow. This element is flanked by direct repeats of 15 nucleotides which occur once in α-tubulin mRNA. These 15 nucleotides are underlined. The sequence of this inserted element and direct repeats is shown in Fig. 4. *b*, Positions and sequences of introns found in the α-tubulin gene ($\lambda\alpha$T14). Sequences of introns are presented with surrounding coding regions. The introns are bracketed. Approximately 60% of the large intron IVS1 sequence has been determined.

DNAs contained sequences complementary to mRNA extracted from rat neuroblastoma cells (line B50; not shown). The RNA/DNA duplex arm of the R-loop measured 1,510 ± 130 nucleotides in length, the same approximate length as the α-tubulin mRNA. Second, the R-loop structures did not reveal the presence of long introns in either duplex. However, in $\lambda\alpha$T14 we frequently observed a cross-over of the two arms of the R-loop at a site ~540 nucleotides from one end. This would be consistent with the presence of a short intron.

Heteroduplex analysis of $\lambda\alpha$T14 and $\lambda\alpha$T15 showed that the two inserted rat DNA segments contained 1,590 ± 110 nucleotides of sequence homology, just sufficient to encompass the total α-tubulin mRNA sequences (Fig. 2*a*). The homologous sequences were not interrupted by long segments of nonhomologous DNA and were flanked by single-stranded loops which did not hybridize to one another in these mounting conditions. In the heteroduplexes of $\lambda\alpha$T14 and $\lambda\alpha$T15 two small knobs were observed at reproducible positions along the duplex segments. Similar knobs have previously been observed for small deletions or insertions in heteroduplexes and suggest that the region of homology is interrupted by a few short segments of nonhomologous sequence. In 5% of the heteroduplexes a single-strand loop of about 1,500 nucleotides was formed at one end of the duplex, suggesting the presence of a larger intron.

Restriction endonuclease cleavage maps were prepared for both $\lambda\alpha$T14 and $\lambda\alpha$T15 phage DNAs by Southern blot analysis using probes similar to those described for Fig. 1. This mapped the α-tubulin related sequences in the phage DNAs to positions adjacent to the longer arm of the Charon 4A vector, and oriented these sequences as indicated in Fig. 2*b*.

DNA sequence of a rat α-tubulin gene

The nucleotide sequence of the α-tubulin related portion of both $\lambda\alpha$T14 and $\lambda\alpha$T15 recombinants was determined by the chemical cleavage method of Maxam and Gilbert[16]. Initially DNA sequencing was done by directly labelling termini in recombinant phage DNA. Subsequently, portions of the α-tubulin related sequences in both $\lambda\alpha$T14 and $\lambda\alpha$T15 DNA were subcloned into the plasmid pBR325 for further sequencing. In total, ~6,000 nucleotides of sequence were determined and over 80% was read from either both strands or from one strand terminally labelled at different cleavage sites.

Recombinant $\lambda\alpha$T14 probably contains sequences specifying an expressed α-tubulin gene of the rat. The nucleotide sequence of this gene is compared with that of the previously sequenced rat α-tubulin cDNA, pILαT1, in Fig. 3*a*. All of the rat α-tubulin mRNA sequence is found in $\lambda\alpha$T14 but is interrupted by three introns. In Fig. 3*a* the positions of the introns are denoted and the sequence of part or all of each intron is given in Fig. 3*b*. All three introns have consensus splicing sequences at their 5′ and 3′ boundaries, permitting assignment of their position in the coding sequence of α-tubulin[17]. Twenty-eight nucleotides upstream from sequences complementary to the suggested 5′ end of the α-tubulin mRNA, is the sequence TATAA-, the expected TATA or Goldberg–Hogness sequence[18]. In preliminary experiments, this DNA segment has been tested for transcription template activity in the whole cell extract system[19]. The length of RNA polymerase II runoff transcripts suggests that the *in vitro* system initiates RNA at a position near or identical to that of the 5′ end of the mRNA, consistent with this being the initiation site of α-tubulin mRNA synthesis.

DNA sequence of a rat α-tubulin pseudogene

Recombinant $\lambda\alpha$T15 contains an interesting α-tubulin pseudogene. First, the α-tubulin related sequences in this phage are remarkably similar to those of the cDNA clone, pILαT1 (see Fig. 3*a*) and therefore to the α-tubulin mRNA. As in the mRNA, sequences corresponding to all three introns in the expressed gene have been precisely excised from the α-tubulin related sequences in $\lambda\alpha$T15. In addition, this gene is homologous to sequences at the 5′ and 3′ termini of the α-tubulin mRNA but the homology to the expressed gene ends precisely at the limits of the mRNA. For example, sequences complementary to the suggested 5′ end of α-tubulin mRNA are present in $\lambda\alpha$ T15 and $\lambda\alpha$ T14 but sequences immediately upstream from this site are totally different (shown in Fig. 3*a*). In the $\lambda\alpha$T15 case, 14 nucleotides of sequence immediately 5′ to the mRNA-related sequences are directly duplicated at the 3′ boundary of the mRNA-related sequences. A short poly (dA) tract is present at the 3′ terminus of the mRNA related sequences in $\lambda\alpha$T15, immediately preceding and included in the second copy of the 14-nucleotide direct repeat. There is no significant homology between $\lambda\alpha$T15 and $\lambda\alpha$T14 DNAs downstream from this position. Some 140 and 540 nucleotides of sequence have been determined 5′ and 3′, respectively, of the α-tubulin related sequences in $\lambda\alpha$T15 DNA.

DNA sequence of a rat dispersed repetitive element interrupting the rat α-tubulin pseudogene

The α-tubulin related sequences in the pseudogene are not completely colinear with α-tubulin cDNA; they are interrupted by a 110-nucleotide segment and a duplication of 15 nucleotides of tubulin sequence at position 1,119 (denoted in Fig. 3*a*). Figure 4 shows the sequence of the 110-nucleotide segment, and the flanking 15-nucleotide direct repeats. This sequence has several features common among dispersed repetitive elements, particularly the Alu-like elements of human, mouse and

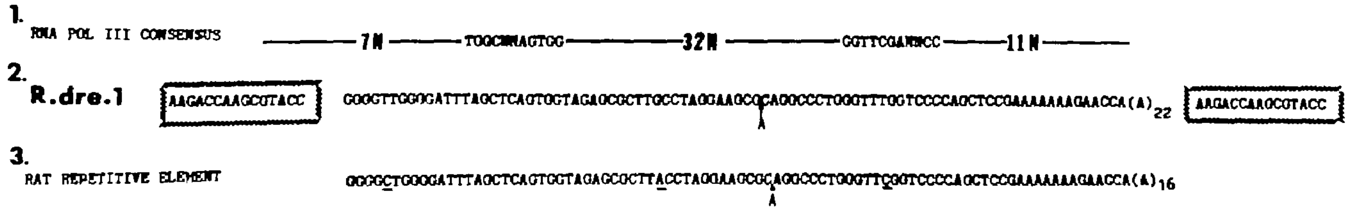

1.
RNA POL III CONSENSUS
7N
TGGCNNAGTGG
32N
GGTTCGANNCC
11N
2.
R.dre.1
AAGACCAAGCGTACC
GGGGTTGGGGATTTAGCTCAGTGGTAGAGCGCTTGCCTAGGAAGCGCAGGCCCTGGGTTTGGTCCCCAGCTCCGAAAAAAAGAACCA(A)22
A
AAGACCAAGCGTACC
3.
RAT REPETITIVE ELEMENT
GGGGCTGGGGATTTAGCTCAGTGGTAGAGCGCTTACCTAGGAAGCGCAGGCCCTGGGTTCGGTCCCCAGCTCCGAAAAAAAGAACCA(A)16
A

Fig. 4 Relationship of repetitive element (termed R.dre.1) contained in the α-tubulin pseudogene. Two-dimensional matrices were used to align the sequence of R.dre.1 with sequences of a closely related element found as the third member of a tandem array of three repetitive sequences located in an intron of rat growth hormone[21]. A second such element (not shown), found several kilobase pairs upstream from the α-tubulin pseudogene, shows a comparable relatedness to R.dre.1. Similar alignments were attempted with consensus sequences for human Alu-like elements, rat 4.5S RNA and rat 7S RNA[42] with only marginal success. These elements are not closely related to R.dre.1. The top line of the figure shows the consensus RNA polIII promoter elements[20] derived from a compilation of tRNA sequences and present in all DNAs thought to be transcribed by this polymerase. In all the comparisons mismatches are underlined, deletions are represented by (−) and insertions are designated.

Chinese hamster DNAs[10]. The 5′ portion of the 110-nucleotide segment contains a consensus RNA polymerase III initiation sequence[20] while the 3′ portion has the customary A-rich sequence. A specific probe for this 110-nucleotide segment hybridizes as a smear to a Southern blot of an *Eco*RI digest of rat DNA. In addition, partial sequence data have revealed the presence of another such element upstream from the pseudogene in λαT15. Barta *et al.*[21] have reported the sequence of an array of repetitive elements in an intron of a rat growth hormone gene. These repetitive elements occur as a tandem array of three related segments. The sequence of one component of this array is also shown in Fig. 4 and only differs in three positions from the element in the rat α-tubulin pseudogene. Clearly, the 110-nucleotide segment, designated R.dre.1 (rat dispersed repetitive element 1), is a member of a rat dispersed repetitive element family and is structurally related to the Alu-like elements.

Discussion

The sequence reported here from λαT14 is the only example of a fully characterized α-tubulin gene which is expressed. We propose that this is a functional gene because: (1) its exon sequences are identical to those of the previously reported α-tubulin cDNA and (2) the only other closely homologous 3′-untranslated sequence in rat is part of an α-tubulin pseudogene. It is likely that there are at least two α-tubulin genes expressed in rat cells, as a second partial cDNA sequence has been reported[3]. However, S_1 nuclease digestions of RNA/DNA hybrids have shown that the α-tubulin gene described here accounts for a large fraction of the α-tubulin mRNA in rat brain as well as in neuroblastoma, fibroblast and glioma cells in culture (I.L. and P.A.S., unpublished observations).

The active rat α-tubulin gene contains three introns, all begin and end in the consensus splicing sequences[17] GT------CAG. The first intron of ~1,770 nucleotides interrupts the coding sequence after the initiation codon ATG, is a class 0 intron[17] and delineates a protein domain of one amino acid. The second intron of 130 nucleotides interrupts codon 76 after the first nucleotide and is thus a class 1 intron. The third intron of 165 nucleotides falls between codons 125 and 126 and is thus another class 0 intron. In view of the limited information about the tertiary structure of tubulin, it is not possible to relate gene structure with protein structure.

Two human DNA segments containing α-tubulin related sequences have been characterized by electron microscopy[5]. It is not clear whether these human DNA segments are expressed α-tubulin genes. These segments hybridize to a chicken cDNA segment but not to human cellular mRNA; both segments have two introns in roughly the same positions as those of the first two introns in the rat gene. However, one segment clearly has a third long intron in the carboxyl-terminal portion of the cDNA which is not present in this rat gene.

Tubulin is a ubiquitous eukaryotic protein and comparison of the number and positions of α-tubulin gene introns in species from different kingdoms might suggest when introns and RNA splicing developed during evolution. A similar comparison has been partially made for actin genes with bewildering results. Intron positions in yeast and *Drosophila* actin genes are unique, while intron positions in sea urchin and vertebrates (both deuterostomes) are frequently conserved[22]. Interestingly, actin genes of vertebrates and soybean have an intron position in common, suggesting that the primordial gene contained this intron[22]. Whether the highly conserved α-tubulin genes will have a similar diversity of intron positions remains to be determined.

As α-tubulin is a major cellular protein, its transcription initiation site should be a strong constitutive promoter. The sequence around the promoter site conforms well with consensus sequences derived from comparison of other sites[23]: GTATATAAGG is found at −31 and GGACCAA is positioned at −77. This DNA segment is active when added as template to a whole cell extract (I.L., unpublished results) but not very active. At the 3′ end of the gene, polyadenylation occurs somewhere in a tract of 3A residues following the customary AATAAA[24].

The intracellular level of α-tubulin mRNA is apparently regulated by the concentration of free tubulin protein[25,26]. The mechanism of this regulation whether transcription, RNA stability or RNA processing is not known. It should be possible to investigate this autoregulation of α-tubulin protein synthesis with probes prepared from the sequenced expressed gene. In addition, sequences responsible for α-tubulin mRNA synthesis and regulation can be defined by *in vitro* modification of the α-tubulin gene and transfection of cells.

The only other cellular DNA sequence highly homologous to the rat α-tubulin cDNA is a pseudogene. This pseudogene was undoubtedly formed by copying of a mature mRNA transcribed from the expressed α-tubulin gene. All sequences corresponding to introns have been precisely deleted, the pseudogene sequences commence at the 5′ end of the mRNA and terminate at or in the poly(A) tract forming the 3′ end of the mRNA. In addition, a direct repeat of 14 nucleotides flanks the precise 3′ and 5′ ends of the pseudogene. This structure strongly suggests that an α-tubulin mRNA was copied into DNA sequence and inserted into a cellular sequence creating the 14 nucleotide direct repeat. Since the pseudogene should be neutral in terms of genetic selection, and differs from the expressed gene by 38 point substitutions in 1,646 positions, the time of its creation can be estimated[27]. Neutral mutations are fixed in the germ line of mammals at a rate of 0.7% per million years. Thus the 2.3% divergence of the pseudogene suggests that it arose $3\text{-}5\times10^6$ yr ago in the germ line of rat. This is well after rat and mouse diverged (30×10^6 yr)[28] and suggests that species even more closely related to rat may not contain this pseudogene.

Evidence for RNA intermediates in the generation of a subclass of pseudogenes has been previously obtained. Pseudogenes related to small nuclear RNAs are characterized by direct repeats that immediately bracket RNA related sequences[9]. Pseudogenes of α-globin of mouse[29] and human λ immunoglobulin[8] are also thought to be RNA derived as they lack introns and the latter terminates in a poly(A) tract. In these two cases, pseudogene sequences related to the expressed loci extend beyond the 5′ terminus of the differentiated cell mRNA. This predicts that an aberrant transcript was used as template in generating these pseudogenes. Two (human) β-tubulin processed pseudogene sequences have also been described[30,31]; each seems to span the amino acid coding sequences and terminates in a poly(A) tract. However, the structures of expressed β-tubulin genes are not known and limit further comparison. In all four cases, the pseudogene sequences are flanked by direct repeats.

A rat dispersed repetitive element is present in the α-tubulin pseudogene sequences. This element (R.dre.1) could have been inserted either in an expressed locus or after the RNA intermediate was used in the creation of the characterized pseudogene. In either case, it is likely that insertion of the repetitive element inactivated the gene and the total began evolving as a neutral sequence. Thus, R.dre.1 should have diverged from its progenitor sequences by less than 2.3%, the divergence of the pseudogene sequence in which it is found. Dispersed repetitive elements almost identical to R.dre.1 have been sequenced by others. An intron of rat growth hormone gene contains a 104-nucleotide segment which only differs in three positions from the repetitive element in the pseudogene (see Fig. 4)[21]. More interestingly, five members of a rat repetitive element family that hybridize to a brain-specific RNA of 160 nucleotides are similarly related to the R.dre.1 element[32]. The function of the 160-nucleotide brain-specific RNA is not known. Sequences related to R.dre.1 are also found in brain-specific hnRNA suggesting that their expression may be specific for certain differentiated cells. This has led to the proposal that these elements be called 'identifier sequences (ID)'[32]. Since the repetitive element in the pseudogene is mobile, any differentiated cell-specific transcription would suggest specific insertion into transcription units only expressed in that cell type. How this specificity could be recorded in germ cells is unclear. The fact that seven rat elements related to R.dre.1 are almost identical in sequence implies that this family of 100,000 dispersed elements is more highly conserved than most families of dispersed repetitive elements. Whether this reflects their recent creation or a high frequency of gene conversion remains to be determined. Given the short period of time that R.dre.1 has had to diverge in sequence, it is likely that these elements are very similar in sequence to that of the hypothetical template RNA. Identification of this template RNA is critical in advancing our understanding of these processes.

Dispersed repetitive elements have been most extensively studied in human DNA where 500,000 copies of a family of related sequences, 300 nucleotides in length, are found, the Alu family of dispersed repetitive elements[10]. About 80% of the members of this family are bracketed by direct repeats of 7–20 nucleotides. The hallmarks of Alu-like elements are nucleotide sequences characteristic of the RNA polymerase III consensus promoter in the 5′ portion and sequences rich in poly(A) tracts in the 3′ portion of the element[10]. Many of these elements are templates for *in vitro* transcription by RNA polymerase III[33]. The RNA product is typically initiated at the 5′ boundary of the repetitive element immediately down stream from the direct repeat. RNA polymerase III consensus sequences are clearly present in the repetitive element (R.dre.1) in the rat α-tubulin pseudogene (Fig. 4). A duplication of 15-nucleotides flanks R.dre.1 in the pseudogene. This duplication probably occurred during insertion of the repetitive element. A similar argument has previously been made for a direct repeat flanking a repetitive element in a monkey satellite sequence[34]. Direct repeats at the site of insertion are characteristic of mobile elements such as transposons in bacteria, ty elements in yeast, *copia* elements in *Drosophila* and retroviruses in mammals[35]. Generation of direct repeats of 7–20 nucleotides is not characteristic of integration of DNA fragments during DNA transformation of mammalian cells[36]. This argues strongly for an active integration process in the mobility of Alu-like repetitive elements.

The striking parallels in the structure of the α-tubulin pseudogene and the rat repetitive element inserted within it, suggest that the two sequences were generated by the same process, a process that (1) copies RNA sequences into DNA sequences, (2) creates direct repeats on insertion, (3) retains the colinearity of inserted sequence and RNA intermediate, and (4) perhaps creates or requires an adenine-rich tract at the 3′ terminus of the RNA or DNA intermediate. Given that there are 500,000 dispersed repetitive elements in human DNA, insertion of small repetitive elements in the germ line of mammals must not be uncommon. It seems reasonable to propose that pseudogenes of mRNAs are generated by the occasional utilization of mRNA substrates by the process which disperses Alu-like elements. Several β-tubulin pseudogenes in man and probably at least three of the α-tubulin related segments in rat (I.L. and P.A.S. unpublished observations) are processed pseudogenes. Whether tubulin mRNAs are special in their propensity to be duplicated and inserted by this mechanism is not clear. However, if they are typical of mRNAs in general, account for 1% of the total mRNA in a given tissue and are converted into processed pseudogenes at a rate of 1 per 3×10^6 yr, it would appear that the germ line of mammals might be increased by 100 such pseudogenes in this period, not a negligible process when considering alterations in the genetic composition of a species.

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LETTERS TO NATURE

Lyα absorption at a high velocity in NGC1275

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The peculiar galaxy NGC1275 = Per A = 3C84 remains a problem in the classification of extragalactic sources despite intensive study in all available wavebands. Included in Seyfert's original list[1], it was described as a Seyfert 2 by Khachikian and Weedman[2] on the basis of observed emission line profiles. Later Weedman[3], however, declined to classify NGC1275 and most recently Véron[4] has suggested that on the basis of its emission line spectrum, variability and radio and polarimetric properties we should regard NGC1275 as a (rather anomalous) BL Lac object. We present here results of IUE spectrophotometry of NGC1275 which show an absorption feature identified with Lyα at a velocity of 8,200 km s^{-1}. We attribute the origin of this feature to the intervening galaxy responsible for the high velocity emission filaments seen around NGC1275. Profile fitting yields a neutral hydrogen column density in the absorbing medium $N_{\mathrm{H}} \sim 1.8 \times 10^{20}$ atoms cm^{-2}. Combining this result with available 21-cm absorption data yields a spin temperature $T_s \sim$ 52 K for the absorbing gas, typical of that in interstellar H I clouds seen in our Galaxy. The implications of these results are discussed.

In addition to the nuclear emission line spectrum Minkowski[5] discovered two discrete emission line systems at 5,300 km s^{-1} and 8,200 km s^{-1} in the network of filaments surrounding NGC1275. The low velocity (LV) system, at the intrinsic redshift of NGC1275 itself, is roughly symmetrically distributed about the galaxy, while the high velocity (HV) filaments are seen only to the north and west of the nucleus[6-10]. The LV system is thought to arise in condensations in an accretion flow of intracluster gas onto NGC1275 [6], excited either by collisional ionization from shocks generated by colliding clouds or by photoionization by the Seyfert nucleus. Observations of the core of the Perseus cluster with the High Resolution Imager on the Einstein Observatory in an effective range 0.7–3 keV also show evidence for a cooling intracluster flow[11], although the relation between the optical and X-ray emission is not fully understood. In contrast to the origin of the LV system, a substantial body of evidence now exists which supports the suggestion that the HV system arises in an infalling late spiral seen along the line of sight to NGC1275 itself[7]. Observations in favour of this hypothesis include: (1) A velocity pattern over the system of HV filaments indicative of a rotating spiral seen almost edge-on[8]. (2) Obscuration of the underlying LV continuum and emission in the HV system are highly spatially correlated. In addition, the Balmer decrement seen in the LV system is much steeper than that seen in the HV system, implying that the latter is intervening[7]. (3) Line strengths in the HV system are typical of those seen in low density, high excitation H II regions excited by stars in the outer regions of late-type galaxies, namely H $\gg$[N II], [O III]$>$Hβ, [N II]$\sim$[S II], [O II]λ3,729$>$[O II]λ3,726. In particular, the low [N II]:[S II] ratio implies a low mass of gas recycled by nucleogenesis in the intervening galaxy[7]. (4) The velocity width of the lines, the Hβ luminosity and the spatial distribution of excitation level in the HV system are consistent with those seen in spirals[6]. (5) 21-cm radio observations show narrow absorption typical of galactic clouds against the nuclear radio source in NGC1275 at a velocity of 8,200 km s^{-1} (refs 12–14).

We have studied three spectra of NGC1275 obtained with the SWP camera aboard the IUE satellite[15]. The wavelength range covered by these spectra is 1,150–1,950 Å; details of the individual integrations are given in Table 1. The absorption feature identified with Lyα at 8,200 km s^{-1} was clearly seen in all three spectra, but only SWP 3452 and SWP 4658 were included in the subsequent analysis due to the poor signal-to-noise ratio in SWP 3430. This was due not only to the shorter integration time but also to the intrinsic weakness of the continuum source in NGC1275 at the epoch of this image (see Table 1). Variability in both the continuum and nuclear emission line spectrum in NGC1275 is rapid and complex, and will be discussed elsewhere (S.A.B. *et al.* in preparation).

The spectra were extracted from the geometrically and photometrically corrected (second file) image using the high resolution package developed by Snijders and Adams[16]. The wavelength scales in individual spectra differed by $<$1 Å and after averaging, the resulting absorption profile was modelled using a curve of growth analysis code developed for use in modelling interstellar line profiles[17]. Only data points to the red of the central absorption velocity were used as the blue wing of the absorption feature was heavily contaminated by Lyα emission at 5,200 km s^{-1} associated with the non-thermal Seyfert nucleus. To facilitate comparison with the model profiles the red wing data were reflected about the central absorption velocity (see Fig. 1). Profiles were generated for a range of H I column densities 0.4–4$\times 10^{20}$ atoms cm^{-2}, that is well on the damping part of the curve of growth, and were convolved with the IUE instrumental profile at 1,240 Å, which is approximated by a gaussian of FWHM 1,000 km s^{-1}, before comparison with the observed profile.

Figure 1 shows the optimum model profile together with the observed data. The data were normalized to the observed continuum and the abscissa shows velocity in the observer's frame. The feature at $v \sim 11{,}500$ km s^{-1} (and reflected at $v \sim$ 4,900 km s^{-1}) is due to Si II $\lambda\lambda$1,260, 1,264 Å galactic absorption.

The optimal fit was found to correspond to a H I column density of $N_{\mathrm{H\,I}} = 1.8\ (+1.0, -0.8) \times 10^{20}$ atoms cm^{-2}. De Young *et al.*[14] have derived $N_{\mathrm{H\,I}} = 3.5 \times 10^{18}\ T_s$ atoms cm^{-2} K, where T_s is the spin temperature of the absorbing material, by study of the 21-cm absorption in the intervening galaxy and combining this with our result we find $T_s = 52\ (+28, -24)$ K. If we assume for the absorbing cloud a gas kinetic pressure $NkT = 2 \times 10^3\ k$ cm^{-3} K as determined for interstellar clouds in the Galaxy[18], then the implied density is $n_{\mathrm{H}} \sim 35$ cm^{-3}. These values for both spin temperature and gas density lie within the limits $T_s < 300$ K and $10 < n_{\mathrm{H}} < 100$ cm^{-3} set by Romney[12] for the 21-cm absorbing medium using 21-cm techniques.

Comparing the observed column density with the derived particle density in the cloud, we can estimate a value for the

Table 1 Short wavelength IUE spectra of NGC1275

Image No.	Date year/day	Exposure time (min)	F_λ(1,500 Å) ($\times 10^{-14}$ erg cm^{-2} s^{-1} Å^{-1})
SWP 3430	1978/328	185	0.48 ± 0.07
SWP 3452	1978/329	345	0.81 ± 0.06
SWP 4658	1979/075	250	0.94 ± 0.06

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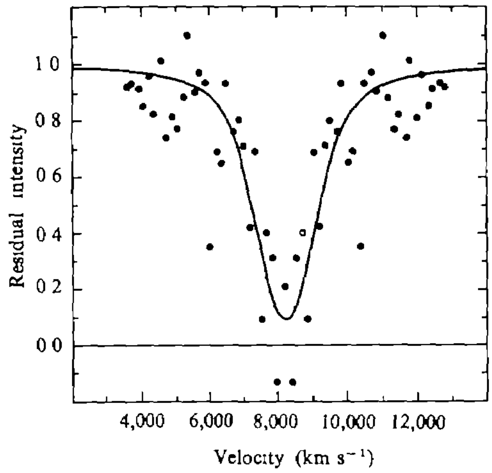

Fig. 1 Absorption due to Lyα at a velocity of 8,200 km s^{-1} in the UV spectrum of NGC1275. The solid line shows the best fit model profile, corresponding to a neutral hydrogen column density $N(\mathrm{H\,I}) = 1.8 \times 10^{20}$ atoms cm^{-2}. Intensity is normalized to the observed continuum and the abscissa shows velocity in the observer's frame. Possible error in centring the absorption feature in velocity space is included in the total error on column density given in the text.

linear dimension of the cloud, $d \sim 2$ pc, consistent with the dimensions of interstellar clouds in our Galaxy. The mean thermal velocity in the cloud is of the order of 1 km s^{-1}, which suggests that the velocity width of 6 km s^{-1} seen in the 21-cm absorption feature arises as a result of turbulent motion within the cloud. This velocity width is again similar to that seen in clouds in the Galaxy.

The position of the cloud in the intervening galaxy can be estimated from the published map of the high velocity filaments[8]. This suggests that the visible galaxy is of the order of 26 kpc in diameter and is inclined at $I \sim 70°$ to the plane of the sky. The nuclear source in NGC1275 lies at the south-east margin of the HV emission, and our line of sight thus passes through the intervening galaxy at angle of about 20° to the disk at a radial distance of ~13 kpc.

The derived density and temperature for the cloud strengthen the conclusion of Kent and Sargent[6] that the cloud must be distant from the nucleus of NGC1275. Scaling their model by our results shows that the cloud would be destroyed by the nuclear X-ray source in NGC1275 unless it were at least 50 kpc from the source, provided the cloud is not heavily shielded against the X-radiation.

The above results thus confirm the hypothesis that the high velocity system of filaments seen about NGC1275 arises in an intervening galaxy distant from and in line of sight to NGC1275 itself.

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SAGE—European ozonesonde comparison

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Between February and December 1979, 30 correlative or 'ground-truth' comparisons were conducted of ozone profiles derived from the satellite sensor SAGE (stratospheric aerosol and gas experiment) and those derived from balloon-borne ozonesondes launched in Europe[1]. The ozonesonde launches took place at Biscarrosse (44.3° N, 1.2° W), Garmisch-Partenkirchen (47.5° N, 11.0° E), Hohenpeissenberg (47.8° N, 11.0° E), and Lindenberg (52.2° N, 14.1° E). Data comparisons were made when the distance between the SAGE profile and ozonesonde launch location was <1,500 km and the time interval between the measurements ≤24 h. This is not an ideal situation but is necessary to accomplish many comparisons without a significant perturbation to the schedules of the ozondesonde launch stations. Even with these large differences in time and space, the comparisons are very good with an average difference between 18 and 28 km of 8.8%. One comparison, however, was within 1 h and a 13 km spatial distance. This was carried out from the 2 April 1979 launch from the ozone station at Garmisch-Partenkirchen. The results are remarkable and together with the general results from the other comparisons are described below.

SAGE uses the technique of limb extinction or solar occultation to infer the vertical distribution of stratospheric ozone. At

Table 1 SAGE-ECC ozone comparisons

Location	Date (1979)	Mean absolute difference (%)
Biscarrosse	7 March	5.5
Biscarrosse	13 March	11.6
Biscarrosse	3 April	12.9
Biscarrosse	3 May	23.0
Biscarrosse	12 June	5.9
Biscarrosse	29 October	4.0
Garmisch	2 April	6.5
Garmisch	10 June	9.2
Hohenpeissenberg	23 February	11.0
Hohenpeissenberg	26 February	6.3
Hohenpeissenberg	28 February	9.5
Hohenpeissenberg	12 March	5.6
Hohenpeissenberg	2 April	6.5
Hohenpeissenberg	4 April	6.9
Hohenpeissenberg	6 April	12.0
Hohenpeissenberg	27 April	9.4
Hohenpeissenberg	30 April	5.7
Hohenpeissenberg	2 May	3.2
Hohenpeissenberg	6 June	14.9
Hohenpeissenberg	11 June	7.5
Hohenpeissenberg	10 October	7.2
Hohenpeissenberg	15 October	7.4
Hohenpeissenberg	17 October	16.3
Hohenpeissenberg	29 October	7.3
Hohenpeissenberg	31 October	6.0
Hohenpeissenberg	24 December	6.5
Hohenpeissenberg	26 December	8.4
Hohenpeissenberg	28 December	14.6
Hohenpeissenberg	31 December	9.4
Lindenberg	28 February	3.4
	Average mean absolute difference	8.8%

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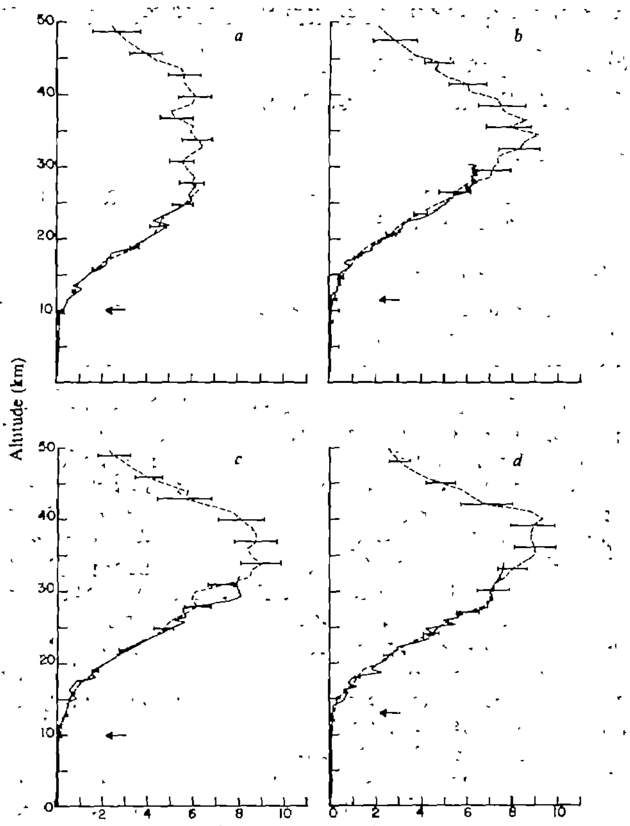

Fig. 1 Ozone comparisons made at four stations in Europe plotted as ozone mixing ratio versus altitude. SAGE data are plotted as the dashed lines and the ozonesonde data as the solid lines. The horizontal lines on the SAGE data indicate the 1σ uncertainties in the SAGE measurements. The horizontal arrow in each comparison plot indicates the altitude of the tropopause. *a*, Lindenberg, 28 February 1979. The separation (Δ*X*) at 20 km altitude between soundings is 828 km. The ECC sonde launch time (T_{ecc}) was 1100 GMT while the SAGE measurement time (T_s) was 0643 GMT. *b*, Garmisch-Partenkirchen, 10 June 1979, $\Delta X = 703$ km; $T_{ecc} = 1716$ GMT; $T_s = 1949$ GMT. *c*, Biscarrosse, 29 October 1979, $\Delta X = 683$ km; $T_{ecc} = 1331$ GMT; $T_s = 1620$ GMT. *d*, Hohenpeissenberg, 31 October 1979; $\Delta X = 871$ km; $T_{ecc} = 0706$ GMT; $T_s = 1638$ GMT.

each spacecraft sunrise or sunset, SAGE acquires the Sun and measures its brightness in four spectral bands in the region 0.385–1.0 μm. The radiances are mathematically inverted as shown by Chu and McCormick[2] to yield profiles of ozone and NO_2 concentration and aerosol extinction with a 1 km vertical resolution on the Earth's horizon. The instrument and experiment are described elsewhere[3]. The ozonesondes used for these comparisons are variations of the two basic sondes, the Brewer–Mast and Komhyr–ECC sonde[4]. They have been shown to operate well to ~30–35 km with an accuracy of 10% (ref. 5).

Table 1 lists the mean absolute differences between SAGE and the European ozonesondes when ozone mixing ratio (p.p.m.v.) is used as the basis for comparison. The mean absolute difference *M* was calculated by

$$M=\frac{1}{N}\sum_{i=1}^{N}\frac{|R_i-r_i|}{(R_i+r_i)/2}$$

where R_i and r_i are the *N* mixing ratios of SAGE and the European ozonesondes at the SAGE altitudes between 18 and 28 km. Figure 1 shows a composite of profiles typical for each station. The horizontal bars on the SAGE data indicate the expected 1σ uncertainties. The tropopause is indicated by an arrow. The comparisons are quite reasonable, especially considering the large differences in time and space. Figure 2*a* shows the 2 April 1979 Garmisch comparison where the time and space differences were very small. Two altitude regions show

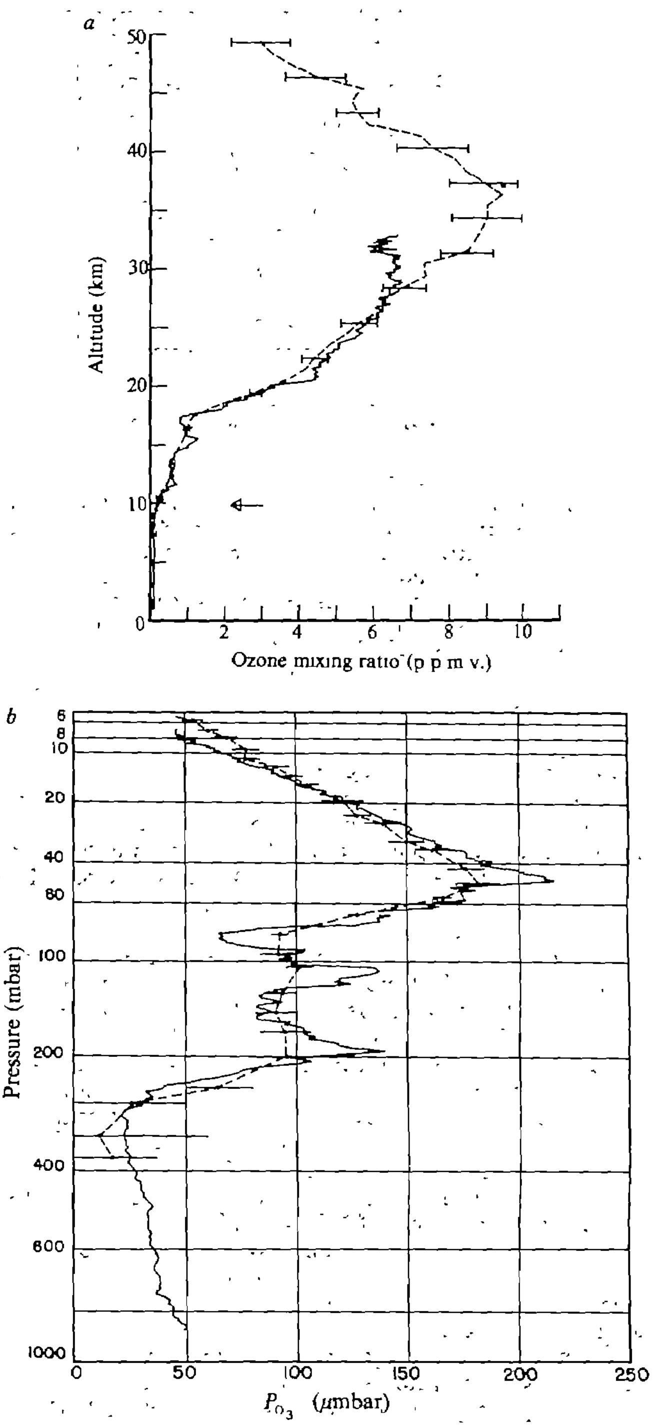

Fig. 2 *a*, Comparison on 2 April 1979, of ozone volume mixing ratio profiles derived from SAGE and an ozonesonde launched from Garmisch–Partenkirchen (47.5° N, 11.0° E). The ECC sonde was launched at 1645 GMT and reached an altitude of 20 km at 1745 GMT. The SAGE measurement was made at 1743 GMT. The horizontal distance between the limb-centroid of the SAGE measurement at 20 km altitude and Garmisch was 13 km. *b*, Comparison as in *a* except ozone is plotted as partial pressure versus pressure altitude.

differences beyond the 1σ expected limits. The fall-off of the ozonesonde data near 30 km is probably due to pump inefficiencies at these altitudes and is characteristic of several ozonesonde soundings. Differences near the tropopause are typical of limb measurement comparisons. Whereas the balloon device samples air as it rises, SAGE integrates its signal over the Earth's limb between the satellite and the Sun. The effective stratospheric pathlength is ~200 km. Over these distances the tropopause and, therefore, the tops of clouds (cirrus and subvisible cirrus) are variable, affecting the measured extinction. In addition, clouds have occasionally been seen to penetrate the local tropopause. Therefore, we feel that SAGE data should not be compared with *in situ* measurements for the first 1 or 2 km above the indicated tropopause. The mean absolute difference over the 18–28 km range of comparison is only 6.5%. Figure 3 repeats this Garmisch comparison in terms of ozone partial pressure versus pressure altitude to >30 km. The effect of horizontal and vertical integration by SAGE is quite evident, but the agreement is remarkable. For example, the ratio of SAGE to ozonesonde values at 13 out of 17 levels between 12.5 and 28.5 km, lies in the range 0.92–1.06. Allowing for uncertainties in the ozone measurements, differences in vertical resolution, time, and space gradients, this agreement is encouraging.

The SAGE ozone data will be archived beginning in summer 1982 at the United States National Space Science Data Center, Greenbelt, Maryland and the World Ozone Data Center, Toronto, Canada.

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Effects of γ radiation on the leaching kinetics of various nuclear waste-form materials

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When a solid used to immobilize high-level nuclear wastes is evaluated with respect to its long-term durability and effectiveness as a barrier against radio-isotope release, the massive doses of ionizing radiation to which the solid will be exposed during storage have to be taken into account. Unlike particle radiation, which can give rise to considerable damage even in dry conditions[1], γ irradiation seems to enhance leachability significantly only when the solid is in contact with an aqueous medium while being irradiated[2]. Even in these conditions studies of the effect of γ irradiation at dose rates similar to those expected in repository conditions show only a modest radiation-induced enhancement in leach rates. This enhancement, expressed in terms of the ratio $R_{irr} = L_I/L_0$ between the leach rates measured in irradiated and non-irradiated conditions, respectively, is typically of the order of 1–4 in the case of borosilicate glasses containing typical commericial as well as defence waste stream compositions[2–4]. We show here that the radiation-induced enhancement of leach rates can be ascribed primarily to radiolytic acidification of the aqueous medium and secondarily to radiolytic formation of complexing species. As a result, wasteforms based on major components such as alumina, which are more susceptible to acid dissolution and to attack by complexing agents than silica, show much larger radiation-induced enhancements of their leach rates than silicate glasses.

A series of waste-form materials including PNL 76–68 borosilicate glass[3], SRL TDS-131 borosilicate glass[4], CU PGM high-silica glass[2] and Synroc-D [5] were leached inside the cavity of a ^{137}Cs γ source. In each case, a sample with a surface area of 2.5×10^{-4} m^2 was placed in a volume of 0.025 l contained in a 0.030-l polypropylene centrifuge tube closed with a screw cap. The γ dose rate was 8.6×10^4 rad h^{-1}, of the same order of magnitude expected at the surface of a waste-form fully loaded with defense waste[4]. Leachants included de-ionized water and a pH 6 0.05 M phosphate buffer. For each combination of solid and leachant two identical experiments were carried out inside the radiation chamber and two outside the chamber in order to evaluate R_{irr}. The temperature in all cases was 25 ± 1 °C. In each case, the leaching period was 3 days, and at the end of this period the leachate was completely removed, analysed and replaced with fresh leachant. This procedure was repeated until five 3-day leaching periods were completed. The experimental error (2σ) in the measurement of leach rates based on Si was up to ±5%, and in the case of other elements up to ±20%. The results showed that the leach rates usually dropped slightly (by <30%) during the first two intervals and became constant throughout the last three intervals. However, R_{irr} did not show significant dependence on time in any of the experiments.

The results of the leach experiments in the cases of the four waste-form materials in water and in a pH 6 phosphate buffer are shown in Table 1. The first part of Table 1, which summarizes the observed radiation-induced leach rate enhancements in water, shows that the enhancements observed in the cases of the three glasses, which range between 1 and 4, are much smaller than those observed in the case of Synroc-D. This is particularly noticeable when one considers the major structure elements, silica in the case of the glasses and alumina in the case of Synroc-D, which determine the long-term corrosion rate of the bulk material. (In the case of the glasses, the small concentration of alumina in the leachates and the relatively high detection limit (0.05 mg l^{-1} Al as compared with 0.005 mg l^{-1} in the cases of the other elements) did not permit an accurate evaluation of R_{irr}). The moderate enhancements found in the case of the glasses agree with previous findings[2–4]. In all cases, the final pH of the irradiated leachates was found to be between 4.0 and 4.3. On the other hand, the pH of the unirradiated leachates remained at the level of 5.7–5.8 corresponding to pure water in equilibrium with atmospheric CO_2.

The second part of Table 1 shows that the enhancements in leach rate become much smaller when the radiation-induced pH decrease is eliminated as a result of buffering the medium at pH 6 using a 0.05 M phosphate buffer. In this case all the enhancements are <2. This is a very strong and direct indication that the radiation-induced acidification of the aqueous medium is the primary cause of the enhancement of leach rates in γ radiation fields.

Based on this conclusion, the large enhancement in leach rates observed in the case of Synroc-D indicates that this material is much less durable in weakly acid media than the glasses. This is directly confirmed by the findings summarized in the third part of Table 1, which show the ratio R_{ac} between the leach rates obtained in high-dilution non-irradiated leach tests carried on the four materials detailed above in solutions buffered at pH 4 and 6, respectively. (The buffer strength in each case was 0.01 M, and the surface-to-volume ratio was 10 m^{-1}; to obtain high accuracy, the tests were carried out at 90 °C with the data corresponding to the period between the second and the fourth days used in Table 1.) The relative sensitivity of the materials to leaching in a weakly acidified medium agrees very well with their sensitivity to leaching in irradiated pure water as documented in the first part of Table 1.

The different alumina content of the four tested materials is probably the reason for the large differences observed in the sensitivity of their leach rates to pH decrease, whether as the result of irradiation or due to the introduction of acid. The solubility of silica is almost completely independent of the pH in the range 2–7 (ref. 6) and is little affected even in more concentrated acids (except HF, H_3PO_4 and their derivatives)[7].

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Table 1 Radiation effects on leaching and related enhancements

	PNL 76–68 ($<0.1\%$ Al_2O_3, 40.0% SiO_2)	SRL TDS-131 (3.7% Al_2O_3, 43.9% SiO_2)	CU PGM (8.1% Al_2O_3, 57.3% SiO_2, 4.4% P_2O_5)	SYNROC-D (19.0% Al_2O_3, 6.75% SiO_2)
R_{irr} (water)				
Si	1.35	3.9	3.5	25
Sr	1.0	1.5		15
Ca	2.3	1.7		36
Na	2.8	1.2	4.2	9
Al				113
R_{irr} (pH 6 buffer)				
Si	1.15	1.2	1.45	1.4
Sr	1.5	1.0		1.0
Ca	0.7	1.2		1.1
Al				1.75
R_{ac} (pH 4/pH 6)				
Si	2.2	11.0	6.5	2553
Sr	12.4	3.9	30	235
Ca	5.6	6.7	7.7	194
Al				66
R_{oxal} (0.05 M/0 01 M)				
Si	2.0	6.9	0.7	3.4
Ca	2.0	7.5	0.7	0.9
Al		6.6	0.9	3.5
Ti	2.3	6.9		11

On the other hand, the solubility of alumina rises very sharply when the pH decreases (by factors of 3, 26 and 1,800 at pH levels of 6, 5 and 4, respectively, relative to the solubility at pH 7)[8]. The sensitivity of silicate solids and glasses[7] to acid dissolution grows rapidly and progressively[9] with the introduction of alumina, and aluminosilicate minerals display a pH dependence almost as sharp as the one observed with pure gibbsite[10].

In the present case, the rise of R_{irr} and R_{ac} with alumina content is roughly exponential, showing a similarity to the data in ref. 9. Focusing again on data based on the major structure elements, it can be seen that in the case of PNL glass, which contains only traces of alumina, the dissolution rate (based on Si) increases only by a factor of 1.35 when leaching is carried out in a radiation field. For SRL and CU glasses, which have an Al_2O_3 content of 4–8%, the corresponding R_{irr} ratios are between 3 and 4, and in the case of Synroc-D (19% alumina) R_{irr} based on Si is 25 but R_{irr} based on Al, the major matrix element in the latter case, is as high as 113. A minor discrepancy is shown by the finding that CU glass is not more sensitive to the presence of acid (as indicated both by R_{irr} and by R_{ac}) than SRL glass despite the higher alumina content of the former. This can be attributed to the presence of a relatively high concentration of P_2O_5 in the case of CU glass alone, as $AlPO_4$ is much less soluble than Al_2O_3 in moderately acidic media[11]. In addition, the high silica content of CU glass is likely to strengthen the matrix against acid attack.

The formation of acidic species on irradiation of aerated water has been observed earlier[3,4], and the pH decrease appears to be larger the higher the radiation dose. Earlier work showed that both nitric acid[3,4] and hydrogen peroxide[3] appear in the irradiated medium. Present findings show the presence of HNO_3, HCOOH, $(COOH)_2$ and H_2O_2 at concentrations of 78, 46, 30 and 16 μmol l^{-1}, respectively, at the end of a 3-day irradiation period in the conditions detailed above. In addition, small amounts of higher organic acids have been detected. The formation of organic acids in aqueous carbon dioxide solutions previously reported[12,13] is likely to result in the present case from reduction of dissolved atmospheric CO_2 by hydrated electrons, which are highly reactive towards CO_2 (ref. 14). The mechanism of HNO_3 formation from dissolved atmospheric N_2 and, possibly, O_2 is less clear, but preliminary doping experiments with alcohols and the observation that hydrated electrons are not reactive towards N_2 (ref. 15) indicate that hydroxyl radicals may be involved.

In any case, the formation of nitric acid may not be very significant in repository conditions in the event that the replenishment of atmospheric gases, after the chamber which contains the waste packages has been sealed, proves to be slow. On the other hand, the fact that at least one half of the total acid content in irradiated water is due to the formation of carboxylic acids from dissolved CO_2 implies that enhanced leaching due to radiolysis may prove to be a persistent effect due to the presence of significant levels of CO_2 and carbonate species in ground-water in general[16]. The extent of carboxylic acid formation in a particular ground-water composition is likely to depend on the concentration of carbonate species as well as on the pH.

Furthermore, the radiation-induced formation of oxalic acd and of other organic acids, as well as the possible formation of aldehyde and glycol[13], raises the possibility that the radiation-induced increase in leach rate is due in part to specific effects of complexing species such as oxalate. It is well-known that glasses undergo accelerated attack in the presence of complexing agents[17].

As the data in the second part of Table 1 demonstrate, leach rates are enhanced by factors ranging up to almost 2 even when the pH decrease is suppressed by means of a buffer. This residual enhancement probably reflects the effect of the formation of oxalate and other complexing species. The fourth part of Table 1 shows the results of measurements of R_{oxal}, the enhancement of leach rates in the presence of oxalate. The measurements were carried out using the same procedure as that used in the non-irradiated leach experiments used in the determination of R_{irr} (25 °C, surface-to-volume ratio $= 10\ m^{-1}$, five 3-day leach periods). R_{oxal} represents the ratio between the leach rates obtained in a 0.05 M pH 5 Na oxalate–oxalic acid buffer and in the same buffer diluted by a factor of 5 with de-ionized water, respectively. The results show significant enhancements in leach rates in all cases, except CU glass, when the samples are exposed to the more concentrated oxalate buffer. The lack of effect in the case of CU glass may be related to the high concentration of silica, which forms a dense network insensitive to complexants. On the other hand, SRL glass and Synroc-D, which exhibit high values of R_{oxal}, have high concentrations of complexable species such as Al and Ti. The build-up of radiation-produced complexing species, in particular oxalate, in ground-water is probably most significant with respect to the leaching of actinides, lanthanides and transition metal ions which are strongly complexed by oxalate.

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The hydrophobic interaction is long range, decaying exponentially with distance

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The attractive interaction between organic nonpolar molecules, such as hydrocarbons, in water is unusually strong. This 'hydrophobic interaction'[1] is responsible for the very low solubility of hydrophobic molecules in water, and has a central role in micelle formation, biological membrane structure, and in determining the conformations of proteins[2,3]. It was once believed that because the interaction is so strong there is a 'hydrophobic bond' associated with it[2,4]; but it is now recognized that the interaction involves the configurational rearrangement of water molecules as two hydrophobic species come together[5–9] and is therefore of longer range than a typical covalent bond. However, there has been no experimental information available concerning the distance dependence and effective range of this interaction. From measurements of the total force as a function of distance between two hydrophobic surfaces immersed in aqueous electrolyte solutions we have determined accurately the attractive component due to the hydrophobic interaction and found that the hydrophobic interaction has the same range as, but is about an order of magnitude stronger than, the van der Waals-dispersion force; and that in the range 0–10 nm it decays exponentially with distance with a decay length of ~1 nm. The results can be roughly extrapolated to molecular interactions and show that the interaction free energy of two hydrophobic solute molecules of radius *R* (nm) in water at 21 °C is approximately given by $\Delta G_H = -40R$ kJ mol^{-1}, which is in agreement with previous estimates. However, the hydrophobic interaction is not due to a 'hydrophobic bond', and its long-range nature has obvious implications for the mechanism and rates of folding as well as the equilibrium conformations of proteins and other macromolecules.

We have measured the forces between two hydrophobic surfaces in aqueous solutions in the distance range $D = 0$ (contact) to $D > 50$ nm.The experimental techniques used were similar to those used previously for measuring the forces between mica surfaces in liquids[10–14], which allow for the force F between two crossed cylinders of mica of radius R to be measured with a distance resolution of about 0.2 nm. In the present experiments the mica surfaces were rendered hydrophobic by the adsorption of a monolayer of the cationic surfactant hexadecyltrimethylammonium bromide, $CH_3\text{-}(CH_2)_{15}\text{-}N(CH_3)_3^{\oplus}Br^{\ominus}$ or CTAB, from solution. At concentrations just below the CMC (10^{-3} M) the positive CTA$^{\oplus}$ group adsorbs onto the negatively charged mica surface as a compact monolayer exposing a hydrophobic surface composed of CH_3 and CH_2 groups[12]. In this range of concentrations the net surface charge on the mica$^{\ominus}$–CTA$^{\oplus}$ surface goes from being negative to positive, passing through its point of zero charge (PZC). Thus in addition to any attractive force between two such monolayer-covered surfaces there is a repulsive electric 'double-layer' force arising from the incomplete charge neutralization at the mica$^{\ominus}$–CTA$^{\oplus}$ interface. We have measured how these forces vary with distance for a range of CTAB concentrations in both distilled water and in NaCl and KBr solutions up to 0.1 M and at various *p*H values in the range 5.4–10.4.

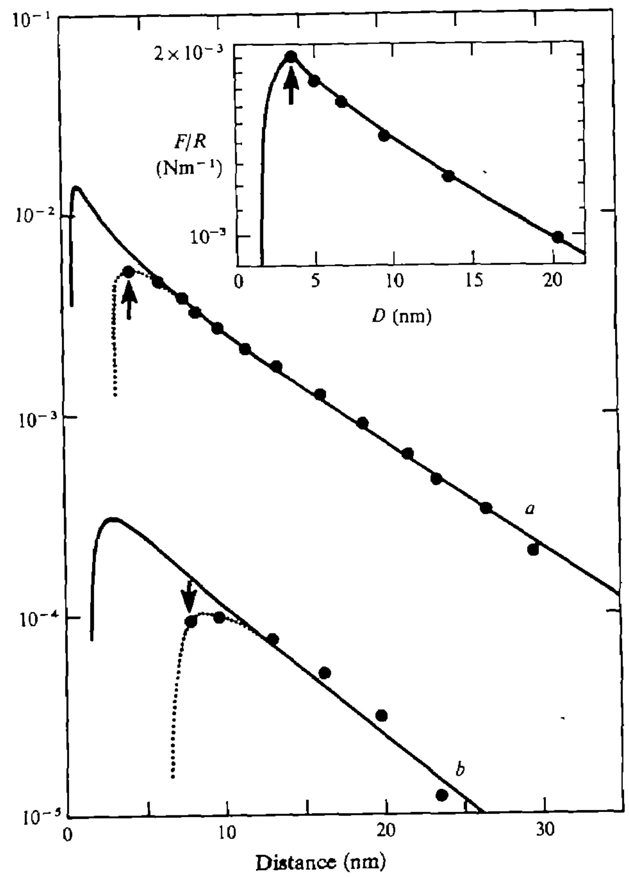

Fig. 1 Measured repulsive force F between two cylindrically curved hydrophobic surfaces (of radii R) as a function of distance D in $\sim10^{-3}$ M NaCl and KBr solutions at 21 °C (plotted as normalized force F/R). The effective surface charge density corresponding to the observed double-layer forces are 1*e* per 5 nm^2 (*a*), and 1*e* per 95 nm^2 (*b*). The solid lines are the theoretical force laws expected from the classical DLVO theory[15,16]. Dotted lines, experiment force laws. Inset: measured force law between uncoated mica surfaces where excellent agreement with the DLVO theory is obtained. Errors in distance measurements are negligible (±0.2 nm); errors in the force are roughly equal to the size of the experimental points, except at smaller distances where the errors are smaller.

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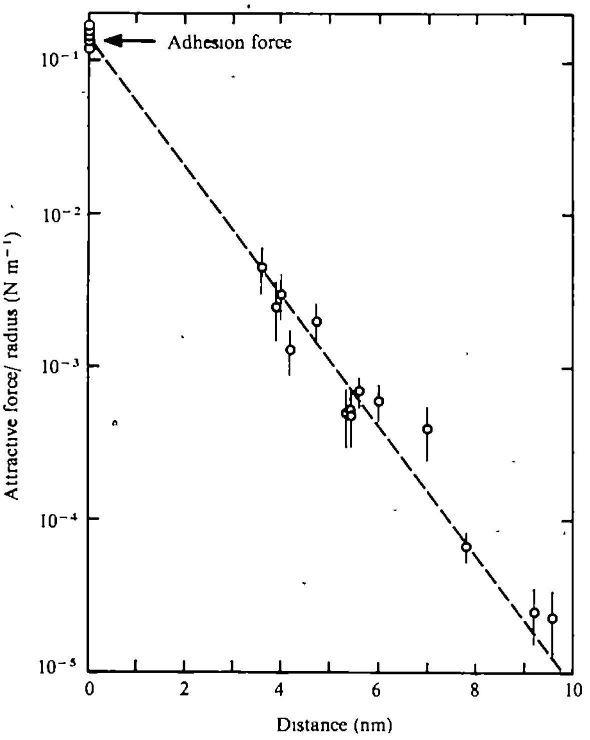

Fig. 2 Attractive force law F_H as a function of distance D as deduced from a wide range of force curves such as those shown in Fig. 1 (and for different values of R between 0.75 and 2.0 cm). Experimental points are obtained from the differences between the measured and theoretical forces in the regions of the observed maxima (arrows in Fig. 1). The hydrophobic interaction decays exponentially out to a separation of about 10 nm, and is given by $F_H/R = 0.14\,e^{-D/1.0}\,N\,m^{-1}$.

Figure 1 shows the measured forces in two instances: high surface charge, and very low surface charge (near the PZC). In each case (and in many others, not shown) the attractive component of the interaction greatly exceeds that expected from the normal van der Waals-dispersion contribution to the total van der Waals + double layer (DLVO) interaction[15,16], which is shown as the solid curves in Fig. 1. This is in marked contrast to similar measurements of forces between uncoated mica surfaces in dilute electrolyte solutions[11,14] where the agreement with the DLVO theory is excellent (Fig. 1 inset).

By subtracting the measured forces from the theoretical curves one can deduce the additional attractive force between the hydrophobic surfaces at different values of D down to the 'force barriers' (arrows in Fig. 1), below which the net force becomes rapidly attractive and the two surfaces jump into monolayer contact at $D = 0$.

The additional attractive (hydrophobic) force is plotted as F_H/R against D in Fig. 2, where we have also included the adhesion or 'pull-off' force at $D = 0$, given by the force needed to separate the two surfaces from adhesive contact. It is apparent that over the distance range 0–10 nm the hydrophobic force-law is well described by an exponential function given by

$$F_H/R = C\,e^{-D/D_0}$$

where $C = 0.14 \pm 0.02\,Nm^{-1}$, and decay length $D_0 = 1.0 \pm 0.1$ nm.

We found that the strength of this interaction is not sensitive to the type and concentration of the electrolyte present, nor to the *p*H. The adhesion force F_H/R at $D = 0$ did, however, increase slightly when the repulsive double-layer forces were weaker, as might be expected. Experiments were also performed with the surfactant concentration progressively diluted to 1/100 of the CMC with no resulting change in the attractive force-law, thus ruling out the possibility that the presence of surfactants is responsible for the extra attraction (through some bridging mechanism involving premicellar aggregates, for example).

The measured attractive force-law is about an order of magnitude larger than the maximum possible van der Waals-dispersion force; and since it decays exponentially with distance rather than as a power law it cannot be attributed to a 'modified' dispersion interaction. It is most likely that this force is the 'hydrophobic interaction'. Although we cannot discuss here all the implications arising from this work or correlate our results with previous experimental and theoretical studies the following three points are notable:

(1) The hydrophobic interaction acts over long-range and cannot be considered as arising from any bond-like association. For two surfaces 50% of the total interaction free energy occurs at distances beyond about two water molecule diameters. The exponential dependence of the interaction has been predicted in recent theories of solvation forces[7,8].

(2) The measured force law given above corresponds to a pair interaction free energy of

$$\begin{aligned}\Delta G_H &= -CRD_0\,e^{-D/D_0}\\ &\approx -84R\,e^{-D/D_0}\,kJ\,mol^{-1}\ (R \text{ in nm})\end{aligned}$$

for two crossed cylinders or for a sphere of radius R near a flat surface, while more generally the value of R above should be replaced by $R_1R_2/(R_1+R_2)$ for two interacting spheres of radii R_1 and R_2. Although there are obvious objections to extrapolating this expression down to molecular radii, it does give surprisingly good values when used to estimate the hydrophobic interaction of small solute molecules in water. Thus for two dissolved methane molecules (hard sphere radius $R = 0.18$ nm)[17–19], two benzene molecules ($R = 0.25$ nm)[17,18], and two cyclohexane molecules ($R = 0.28$ nm)[17,18], the values obtained for the free energy of dimerization ($\Delta G_H \approx -42R$ kJ mol^{-1} at $D = 0$) in each case are $\Delta G_H \approx 7.6$, 10.5 and 11.8 kJ mol^{-1}, respectively, which compare surprisingly well with the various theoretical and experimentally determined values of 7.5–8.8 (refs 5, 6), 8.5–9.6 (refs 9, 20) and 11.3 (ref. 20) kJ mol^{-1}.

(3) The long-range attractive forces measured between surfactant[12] and lipid bilayers[21,22], where both hydrophilic and hydrophobic groups are present at these interfaces, appear to be well described by the van der Waals-dispersion force, suggesting that the hydrophobic interaction is neutralized when the local structure of water molecules is dominated by their interaction with nearby hydrophilic groups.

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Oscillatory spreading explanation of anomalously old uplifted crust near oceanic transforms

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Rocks significantly older than their theoretically predicted age have been recovered in the vicinity of some Atlantic Transform Zones. Rocks of Palaeocene (55–58 Myr) and possibly even of Cretaceous age are found near the Vema Transform, on tectonically uplifted crust which should not be older than 30 Myr. We present here a model which might explain the presence of uplifted, anomalously old crust near the Vema Transform Zone (TZ). The proposed model involves axial rift propagation and reorientation and migration of the transform as a result of a change in the regional stress field. During transform migration a crustal wedge, formerly part of the African plate, is transferred to the South American plate. The ensuing oscillatory spreading can explain the anomalous age of the uplifted crustal block. Compression resulting from rift propagation and transform migration provides a mechanism for crustal uplift.

Two fundamental tenets of the theory of plate tectonics are: (1) the age of the oceanic crust increases with distance from the axis of crustal accretion (the axis of mid-oceanic ridges) where the age is close to zero; and (2) the depth below sea level of the oceanic basement increases proportionally to the square root of the age of the crust, due to cooling and thermal contraction of the oceanic lithosphere during its motion away from the axis of accretion. These two tenets are supported by a wealth of observations obtained from the three major ocean basins. However, exceptions exist. In particular, crustal blocks which have been subjected to intense vertical tectonic movements and reach anomalously shallow depths have been observed in the vicinity of large offset ridge–ridge transform zones in the Atlantic and Indian Oceans[1-3]. Moreover, rocks significantly older than their theoretically predicted age have been reported from the vicinity of some Atlantic transform zones, namely the Atlantis TZ at 29° N (ref. 4), the St Paul TZ to 1° N (ref. 5) and the Vema TZ at 11° N (refs 6–8). No satisfactory explanation has been presented to date, which would reconcile seafloor spreading with the occurrence of old material close to the axis of lithospheric accretion.

The Vema TZ offsets the axis of crustal accretion by ~300 km (Fig. 1). The transform valley is filled with nearly 1 km of sediment; the recent strike-slip transform motion can be traced in the sediment close to the axis of the transform valley[9]. To the south of the transform valley and parallel to it, lies a narrow ridge (transverse ridge) whose summit is shallower than 600 m below sea level[10,11]. The ridge constitutes a major topographic anomaly relative to the predicted depth–age relationship (Fig. 2), and is probably a tectonically uplifted sliver of crust and upper mantle[11,12].

The summit of the uplifted ridge is capped by shallow-water reef limestones, some of which have undergone sub-aereal diagenesis. Palaeofacies studies and age determinations of these limestones indicate that the summit of the uplifted block was above sea level from late Miocene up to middle Pliocene time (from ~10 to 3 Myr BP). It subsequently subsided at an average rate of 0.3 mm yr^{-1}, a rate one order of magnitude faster than predicted by thermal contraction of the plate[8].

Although equatorial magnetic anomalies are not very well developed, they are good enough to estimate spreading rates. Values ranging from 1.1 to 1.4 cm yr^{-1} have been determined from profiles located immediately north of the transform[11,13,14]. Assuming this spreading rate is correct, and that the uplifted crust on the southern side of the transform valley was originally created at the southeastern axial segment of the Mid-Atlantic Ridge, an age of 30–35 Myr can be estimated for the area of maximum uplift on the transverse ridge (Fig. 3). However, deep-sea limestones were recovered from the uplifted block (in addition to the shallow water samples), which have been dated as early Palaeocene (~55–58 Myr BP) on the basis of their fossil plantonic foraminiferal fauna[8]. This anomalously old age is consistent with ages determined from other limestone samples bearing pre-Tertiary fossil crab coprolites of the genus *Parafavreina*[6,7].

It is possible that the anomalously old crust is related to reorientation and migration of the transform in the past. A set of sediment-filled valleys south of, and striking 10°–15° oblique to the Vema Transform (Fig. 1) were identified and interpreted as traces of former transforms by Van Andel *et al.*[11,13]. These fossil transform traces are present only in crust older than ~10 Myr. The different orientation of the former transforms relative to the presently active Vema TZ suggests that a reorientation by 10°–15° of the direction of spreading occurred

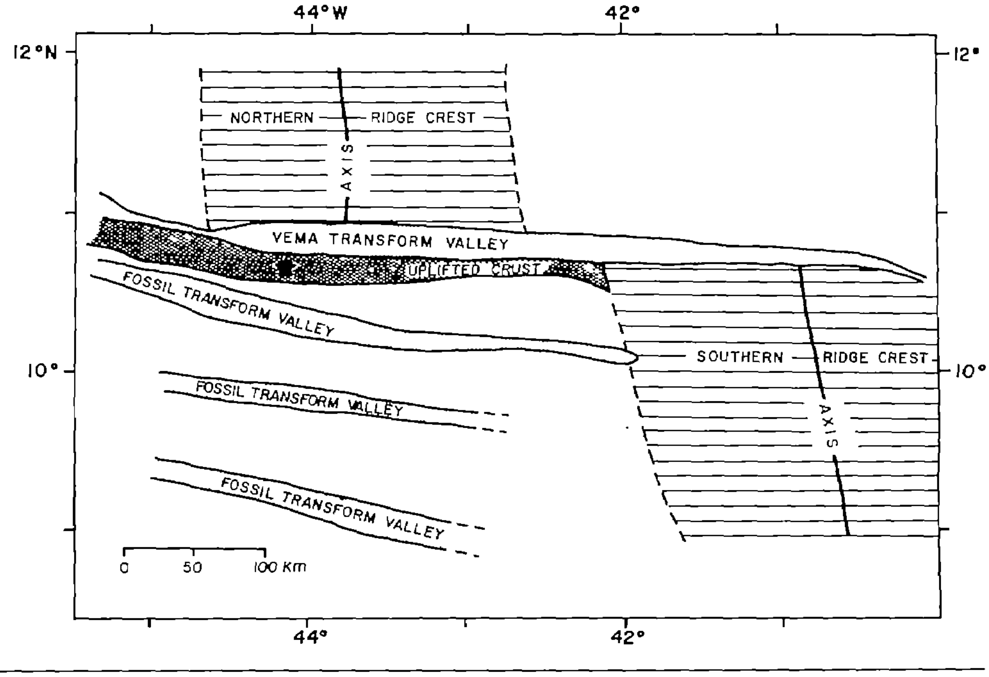

Fig. 1 Primary morphotectonic features at the Vema TZ in the Atlantic, after refs 11, 13. ★, Site of maximum uplift (<600 m below sea level) on traverse ridge.

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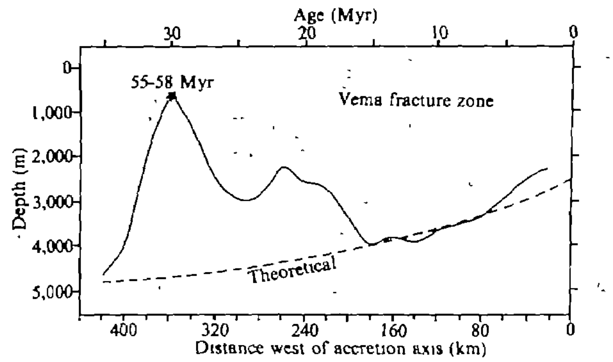

Fig. 2 Topography versus age on a profile immediately south of the Vema Transform valley, compared with the theoretical curve[23] predicted for thermal contraction of the lithosphere. A spreading rate of 1.2 cm yr^{-1} is assumed.

in this area $\sim$10 Myr BP (ref. 13). This reorientation is also suggested by both a change in the trend of the western extension of the Vema fracture zone, and by a corresponding change in strike of the 10-Myr old magnetic anomalies[13].

Based on this information, we propose the following sequence of events to explain the existence of anomalously old and tectonically uplifted crustal blocks along the transform (Fig. 3).

(*a*) Before $\sim$10 Myr ago a ridge–ridge transform zone was active to the south of the present Vema TZ. The strike of the former transform was 10°–15° NW of the present transform strike.

(*b*) A change in spreading direction of 10°–15° occurred, probably related to a reorientation of the lithospheric regional stress field. In response to the changing stress field, the south-east segment of the accretion axis gradually propagated northward. This northward propagation of the axis of spreading might have taken place in a manner similar to that suggested for the Juan de Fuca ridge, the Galapagos rift and the East Pacific Rise[15–18]. According to Fujita and Sleep[19] and Crane and Gallo[20] the magnitude and direction of intraplate stress acting on the ridge–transform system may be the primary cause for the readjustment of a transform and the reorientation of the zone of extension.

(*c*) A new ridge–transform–ridge geometry, similar to that of the present Vema TZ became established $\sim$10 Myr BP.

(*d*) Spreading in an east–west direction has occurred for $\sim$10 Myr. The fossil transform valley can be traced south of the presently active Vema TZ.

We stress that the geometry outlined in the evolutionary scheme of Fig. 3 can be modified without changing the substance of the model. For instance, we have assumed that the change in orientation of the axis of spreading and of the transform pivots around a fixed point at the southern tip of western axis of spreading. This assumption may not be strictly true.

One consequence of the model illustrated by Fig. 3 is that a portion of crust close to the northern side of the former transform boundary moved east-south-east before the reorientation of spreading direction (Fig. 3*a*). After the migration of the transform to the north, this wedge of crust was transferred from the African to the South American plate and thus began to move to the west (Fig. 3*c*, *d*). We refer to this reversal of spreading direction as 'oscillatory spreading', and we suggest that this might explain the presence of anomalously old crust on the southern side of the Vema TZ.

Considering the location and age of the anomalously old rocks, assuming an average spreading rate of 1 cm yr^{-1} or more, and assuming that the present spreading direction became established roughly 10 Myr ago, it follows that the 55–58-Myr old crust cannot be explained simply by one cycle of instantaneous transform reorientation and migration, as depicted in Fig. 3. The simple model of Fig. 3 can explain the 55–58-Myr old crust if there was a transitional period of reorientation and migration of the transform lasting several million years, during which the wedge of crust between the old and the new transform would remain more or less stationary. Alternatively, the anomalous 55–58-Myr old crust might have been involved in more than one stage of migration of the transform by rift propagation. The presence of crust as old as Cretaceous, if confirmed, would support the idea that several transform migration steps have occurred. The existence of at least three fossil transform valleys to the south of the presently active Vema TZ (Fig. 1) suggests indeed that the northward transform migration by rift propagation occurred in more than one step.

The observation that the presently active transform valley is filled by sediments younger than a few million years[11,21] supports a young age for the Vema Transform Valley and is consistent with the suggested model.

Transform reorientation and migration might be responsible also for the vertical tectonic motions which gave rise to the uplifted crust at the Vema TZ. Vertical tectonic movements of crustal blocks can occur in the vicinity of a transform zone due to several distinct processes[12,18,22]. One process would involve small changes in spreading direction, which result either in

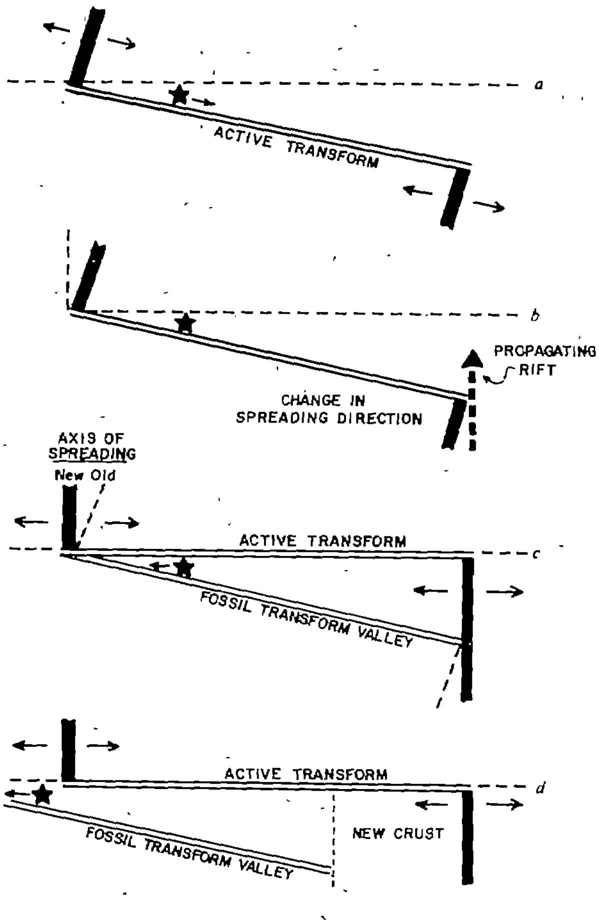

Fig. 3 Proposed model on transform migration and oscillatory spreading. *a*, Former ridge–transform–ridge geometry. Crust at site ★ is part of African plate and moves east-southeast. *b*, A change of 10°–15° in spreading direction occurs. Southern ridge axis starts propagating northward. *c*, Situation 10 Myr ago. Migration and reorientation of transform has taken place. Site ★, now part of South American plate, reverses its motion and starts to move to the west giving rise to oscillatory spreading. *d*, Present situation, site ★ continues to move westward. Fossil transform valley present south of the present transform. To explain the ages obtained from the summit of the uplifted crustal sliver, more than one cycle of transform migration is required. Alternatively a long (several million years) period of transition between stages *a* and *c* is necessary.

compression or extension along the fracture zone. Within our proposed model of rift propagation and transform reorientation and migration, compressional regimes are likely to occur in portions of the crustal wedge situated between the old and the new transform. The wedge should be subjected to maximum compression during the time of transition from the old to the new ridge–transform–ridge geometry (stage *b* in Fig. 3). Crustal uplift might have occurred in response to the compressional regime. Note that the summit of the uplifted block stayed above sea level from at least mid-Miocene (~10 Myr BP) to mid-Pliocene (3 Myr BP) time[8]. These ages are consistent with a model which predicts the subsidence of the block after the present ridge–transform–ridge configuration became established, ~10 Myr BP. Uplift took place before 10 Myr ago.

A possibility to be considered is that a compressional regime develops in front of a propagating rift. In cases where the rift is unable to advance, compressional stresses at its tip may induce vertical movements of the crust. It is possible that during the reorientation of the Vema ridge–transform system, while the south-east axis of accretion was able to propagate northwards, the north-west axis did not propagate to the south but created compression and crustal uplift on its front across the transform. In fact, we note that the uplifted crustal block is located roughly across the transform from the north-west axis of spreading.

A more precise assessment of the processes which led to crustal uplift and presence of anomalously old crust at the Vema TZ may allow us to use the proposed model to explain transform related anomalous ages and vertical tectonics in other areas.

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Fault location by radon and mercury detection at an active volcano in Nicaragua

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The Masaya Caldera Complex, Nicaragua, with the active crater, Santiago (Fig. 1), is the site of present-day basaltic volcanism[1,2]. Some of the faults that bound the caldera and lie within it can be located geologically; others are postulated. A possible older caldera rim lies to the south of the present margin. To investigate whether one can identify faults in this environment of active volcanism, concentrations of radon and mercury in the regolith were determined at 232 selected points in and adjacent to the caldera. Higher concentrations of Hg^0 or Rn or both occurred over 75% of the known structures studied. Some hitherto suspected structures were confirmed while others were not. Lack of a chemical signature may be ascribed to the absence of a structure, inappropriate sampling sites, post-fault lava cover or other sealing of the structure. We show here how the use of Rn and Hg^0 determinations has helped in structural analysis at this active volcano.

Radon was detected using α sensitive film enclosed in Track Etch Detector Cups which were buried at 40-cm depth for ~30 days. Analyses of the tracks on the film were performed by the manufacturer. Radon is reported as tracks per mm^2 per 30 days (0.91 tracks per mm^2 per 30 days = 1 pCi l^{-1} for the allyl deglyol carbonate detector used)[3]. Replicate radon analyses indicated a deviation of 10%, similar to the deviation reported by others[4–6].

Mercury was determined, using a gold film mercury detector (Jerome Instrument Corp., Model 301), in the $<0.5\,\phi$ (0.17 mm) size fraction of the regolith collected from 40 cm depth at each of the Rn locations. The 5% deviation indicated by replicate analyses agrees with that of Cox and Cuff[4].

Traverses (Fig. 1) were made[7] approximately perpendicular to known or suspected faults or fissures, and were usually 150–300 m long with 6 or 7 samples per traverse. Seventeen traverses crossed structures for which geological evidence was strong. Sixteen other traverses crossed postulated structures.

In a previous study[5] Rn was successfully used to delineate fault zones in a known geothermal area, but was of different scale (60–150 m cup spacing) and in different terrain than the one described here. A geothermal resources survey on the north rift zone of Haleakala volcano, Hawaii used a 1–1.5 km sample spacing[4]. Significantly elevated Hg^0 and Rn values marked the

Table 1 Variations in Rn and Hg^0 with nature of the regolith

	Radon (tracks per mm^2 per 30 days)		Hg^0 (p.p.b.)	
Nature of regolith	Geometric mean	s.d.	Geometric mean	s.d.
Recent lava flow	3.3	+2.6 −1.5	21.8	+17.4 −9.7
Old flows with moderate soil cover or ashfall or scoriafall	10.1	+7.1 −4.2	25.3	+15.2 −9.5
Well developed soil, often cultivated	45.1	+94.7 −30.6	35.8	+17.9 −11.9

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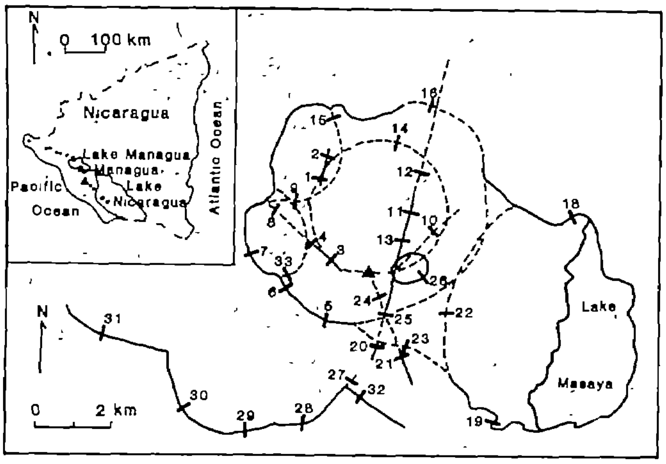

Fig. 1 Partial and simplified map of structures of Masaya Caldera Complex. Solid lines indicate location of faults or fissures known on basis of geology; dashed lines indicate location of postulated faults or fissures; bars indicate location of traverses (not to scale). Numbers of traverses are referred to in text (traverse 17, not shown, lies to north on regional fault). ▲, Location of Santiago crater, site of present-day activity. Inset shows location of Quaternary volcanoes (●) and study area (▲) in Nicaragua.

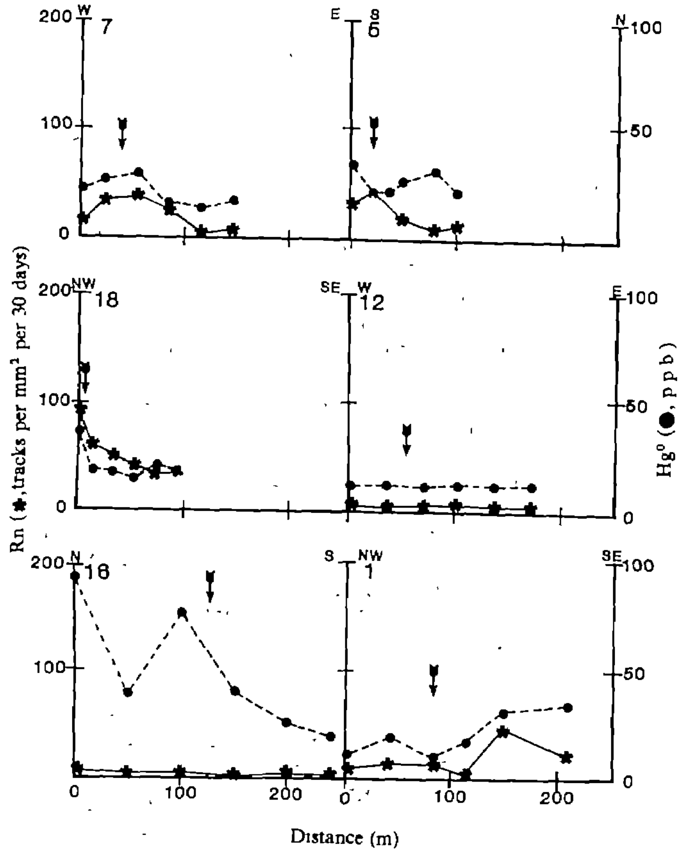

Fig. 2 Rn and Hg^0 signatures from selected traverses (numbers in upper left of each example as in Fig. 1). Arrow indicates location of fault or fissure based on field geology. Individual traverses: 7, Hg^0 and Rn peaks coincide with each other and with projected fault location (caldera fault scarp); 5, Rn and Hg^0 peaks separated by 60 m (caldera fault scarp). 18, Fault probably on the extension of the traverse (caldera fault scarp). 12, Neither Rn or Hg^0 show significant change along traverse (an attempt to locate position of fissure). 16, Hg^0 signature 2 peaks, but no significant Rn peak (hidden caldera fault scarp). 1, Hg^0 peaks and a less marked Rn peak separated by 50 m (an attempt to locate portion of a fissure).

rift zone. The survey reported here examined narrower fault zones and in much greater detail.

Data have been selected from this study to show the variation in Hg^0 and Rn signatures (Fig. 2). Radon and mercury signatures marked the caldera-bounding fault on all traverses across it (5, 6, 7, 16, 18, 19, 23) although detection of two segments where visual expression was lacking (16, 23) was uncertain. The scalloped caldera boundary suggests coalescing collapses rather than a single engulfment[1]. Both Rn and Hg^0 analyses on four of the five traverses crossing presumed locations of such structures (4, 8, 9, 15, 33) indicate that these circular features may exist beneath the covering of recent basaltic lava flows. Circular distribution of active vents in the caldera may outline the trace of a collapse crater[1] but Rn and Hg^0 analyses (3, 14) do not support its existence. A third hypothesis suggests that there may be two overlapping caldera collapse features (J. Garayar, personal communication). The traverse (22) designed to test such a hypothesis did not support its presence. A proto-caldera rim, outside the present scarp, may be represented by a deeply eroded fault scarp. Rn analyses from samples on traverses across this fault (27–32) confirm its presence. Hg^0 analyses were less conclusive. Linear features cross the caldera floor, but prediction of their location, despite surface expression in some instances, met with mixed success. Of 11 traverses across faults or fissures, one (11) failed to indicate a fault, two (4, 25) indicated possible faults and the others (2, 3, 8, 10, 12, 13, 20, and 24) indicated faults in the predicted location. For the 16 traverses over known faults or fissures Rn and Hg^0 both successfully predicted 75% and for the 17 traverses over possible faults Rn successfully predicted 65% and Hg^0 76%.

Radon values (Table 1) are very much larger in areas of well developed soil, as in Hawaii[8], but the ratio of peak to background values is greater in most localities outside the area of well-developed soil. Factors affecting radon concentration include recent lava flows acting as effective barriers to ground gas migration and the fact that the radon content in the tholeiitic lavas is lower than in the more siliceous volcanics in the areas of best soil developed outside the caldera. The high porosity of soils compared with the sparse regolith developed over recent flows allows more effective radon migration and the greater surface area enhances radon emanation. At Masaya Caldera Complex, meaningful measurement of radon in areas of fresh lava flows is feasible, despite the very low peak values.

The Hg^0 content of the fresh lava sampling sites (2, 3, 9, 11–16, 22, 23, 25) (geometric mean of 21.8 p.p.b. (part per 10^9)) is much greater than reported[9] from Icelandic lava, 2–9 p.p.b. Hg^0 data (Table 1) indicate less distinctive populations in the three areas defined by the type of regolith. The ratio of peak to background values is usually smaller in areas of well-developed soil than elsewhere. Secondary soil processes may be important in determining the background variation of Hg^0, as at Long Valley California[10].

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Hidden cues in random-line stereograms

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Vernier acuity shows that the human visual system can interpolate the relative position of a feature with astonishing precision, of the order of a fraction of the distance between neighbouring photoreceptors in the fovea. The remarkable properties of this interpolation process have been the subject of many recent studies[1–9]. A major question that is still unanswered is whether the process is parallel—operating automatically at all times over a large part of the visual field—or serial—operating selectively at isolated locations only when judgments in the hyperacuity range are required (as in a forced choice task). Successful fusion of random-line stereograms having breaks in the vernier acuity range has been previously interpreted[10] to suggest that the interpolation process underlying hyperacuity is parallel and preliminary to stereomatching. Here we demonstrate with computer experiments that vernier cues are not needed to solve the stereomatching problem posed by these stereograms, and we provide psychophysical evidence that human stereopsis probably does not use vernier cues alone to achieve fusion of these random-line stereograms.

Julesz and Spivack's[10] claim that stereo fusion of their random-line stereograms is based on vernier cues alone is apparently the best evidence for the interpolation process being parallel; Fig. 1*a* shows an example of their random-line stereograms. Figure 2 illustrates by a small 4 × 5 array of picture elements, the way in which Fig. 1*a* was generated. Each picture element contains a thin vertical line segment at one of two possible horizontal positions, separated by one-fifth of the picture element diameter. The random selection of the two positions to make the 4×5 array produces five vertical lines through the array with frequent small horizontal breaks. Once the left array is generated in this way, it is copied into the right array where a rectangular region of picture elements at the centre (dotted lines) is shifted horizontally by an integral number of picture element diameters (here the shift is one picture element). Thus the pattern of breaks is correlated in the left and right arrays with zero disparity around the borders and a fixed horizontal disparity in the central region. Figure 1*a* is the same except that the array has 100 × 100 picture elements and the horizontal shift is two picture elements. Similar patterns can also be made with horizontal line grids. The only obvious monocular information is the pattern of minute horizontal breaks occurring randomly in the thin vertical line grids. The stereograms clearly yield stereopsis—the centre square is seen in front of the surround—even with monocular breaks of as little as 16 arc s, which is below the threshold for resolving two lines. Note that the stereomatching problem posed by this type of stereogram is not solvable by simply measuring the position disparity between nearest line segments in the two arrays of the stereo pair. The correct matches in the central region are shifted horizontally by one or more picture elements so the nearest matches are all false targets. Thus, the matching problem can only be solved by taking into account, in some manner, the pattern of breaks in an area. For this reason Julesz and Spivack[10] argued that it is necessary to detect the vernier breaks and match corresponding ones before stereo fusion.

The key question is whether vernier cues are indeed the only monocular information present in this type of stereogram. It has been suggested to us (H. Barlow and W. Richards, personal communications) that receptive fields—for example, centre-surround ganglion cells—each integrating over angular extents of several arc min might detect coarse monocular structures in such stereograms. If so, this structure alone may be sufficient to drive binocular stereomatching without need of vernier precision. Indeed, a recent theory of human stereo vision[11–14] (see also ref. 15) proposes that the two images are filtered through several, roughly bandpass, channels, each with a different centre-surround receptive field size, and that stereomatching is based mainly on zero-crossings (boundaries between regions of positive and negative response) in each of these channels. Essentially all images (for example, random-dot stereograms) when processed in this way, reveal a clear coarse structure (see for example Fig. 3.6 of ref. 13). When the random-line stereogram of Fig. 1*a* is filtered in the same way, coarse monocular features do appear (Fig. 1*b*). The underlying cause of this coarse structure is the variation in spacing between neighbouring lines. While the absolute changes in spacing are small, the percentage change in the line spacing is appreciable. For the patterns described by Julesz and Spivack, the ratio of smallest to largest line spacing is 2:3. Figure 1*b* shows the locations where a centre-surround operator would give positive (white) and negative (black) responses to the random-line pattern shown in Fig. 1*a*. The coarse structure here is well preserved by the photoreceptor sampling at intervals of 30 arc s. Interpolation between these points to increase spatial precision is unnecessary.

Many matching algorithms can successfully use the monocular features provided by the larger operators to solve the stereo correspondence problem. As an example, Fig. 1*c* shows the output obtained with the cooperative algorithm of Marr and Poggio[16,17] when applied to the binary array shown in Fig. 1*b*. It identifies correctly the different disparities of the centre square and the surrounding background. Essentially the same results are obtained with the other stereomatching algorithms developed in our laboratory, when applied to the same filtered images. Thus these random-line stereograms can be solved without need of vernier precision.

At this point, our computer demonstration shows only that non-vernier, monocular cues are present and could be used for successful stereomatching, but the question remains of whether our visual system actually uses them instead of the vernier cues. Psychophysical experiments can be used to test these hypotheses. We have examined the differences expected between systems relying entirely on one or the other approach. As noted above, the coarse structure shown in Fig. 1*b* is due to the large line spacing variation in the patterns. Increasing the line spacing while maintaining a fixed break size reduces the detectability of coarse structure present in the patterns but does not affect the detectability of the vernier breaks (larger spacings should actually improve vernier acuity[6]).

Figure 3 shows a random-line stereogram having twice the line spacing as shown in Fig. 1, but with the same break size and disparity for the central region. This stereo pair is difficult to fuse when viewed at a distance which gives a vernier break size of about 15 arc s while the pair of Fig. 1*a* can be fused easily at that distance. These patterns were computer-generated on a high-resolution cathode ray tube and viewed stereoscopically from a distance of 4 m at which the size of the vernier breaks was ~15 arc s. We tested four subjects in these conditions and for all of them the stereogram of Fig. 3*a* was much more difficult to fuse than Fig. 1*a*; this is consistent with the computer simulations (compare Fig. 1*c* with Fig. 3*c*).

Other psychophysical observations, though less critical, are also consistent with the large channel hypothesis. For example, we expect that (1) random-line stereograms could be fused for very small break sizes if line spacings were made proportionally smaller—fusion would eventually be limited by the loss of contrast as the receptive field extends across more than several

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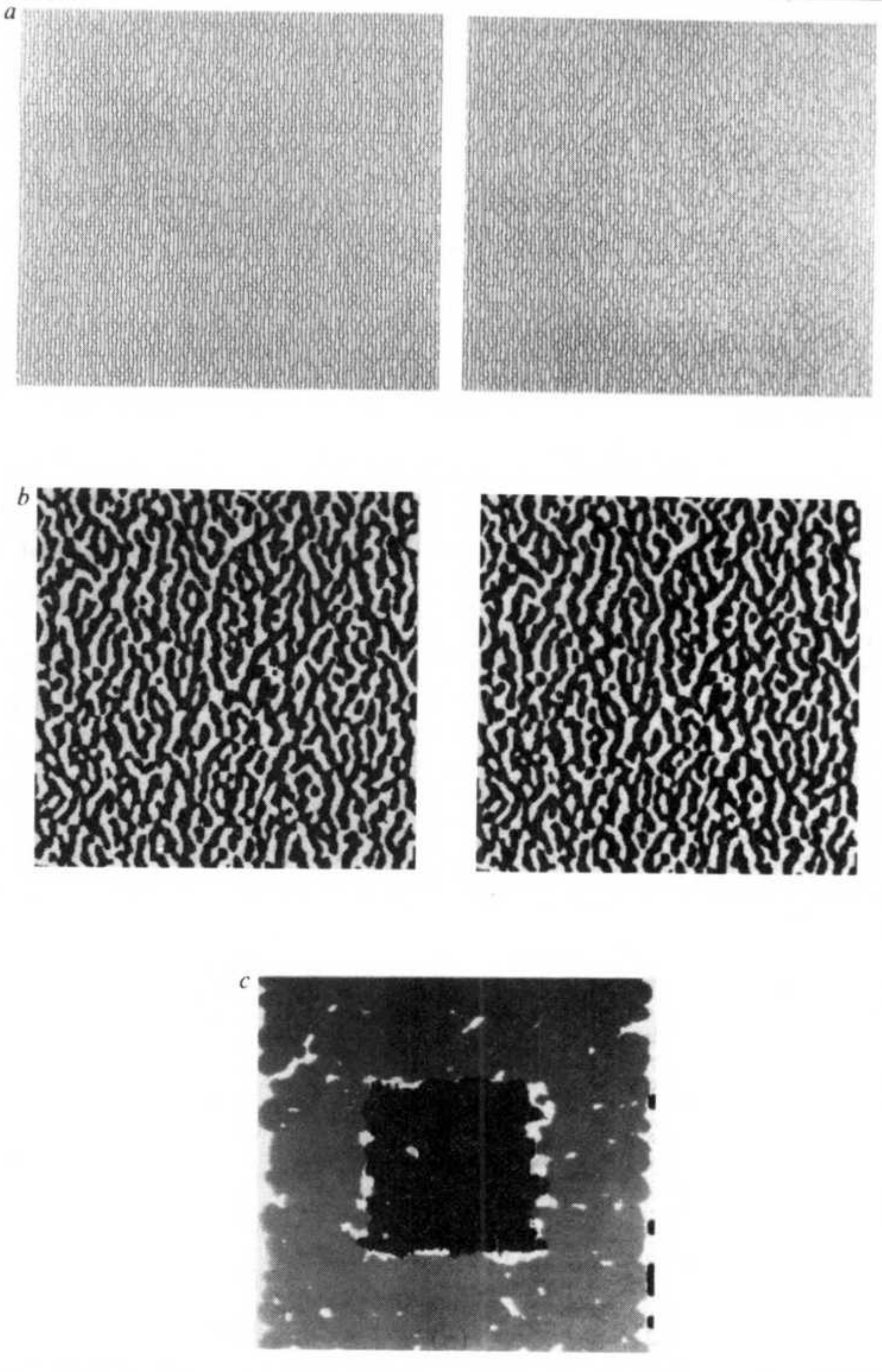

Fig. 1 *a*, Random-line stereograms of thin vertical line segments with small breaks, portraying a centre square in front of the surround. When the patterns as printed here are viewed at 1.4 m, the vernier breaks are ~15 arc s and the minimum line spacing is 1 arc min (the spacing and the diameter of the cones in the human fovea is ~30 arc s). The central square has a disparity relative to the background of 10 times the break size. *b*, The stereogram of *a* filtered through a centre-surround operator consisting of the difference of two gaussians (DOG)[19]. The width of the operator's centre is 16 times the break size in *a*. When *a* is viewed from a distance making the break size 15 arc s, this corresponds to what a filter with a 4-arc min centre would compute—a medium-sized channel as revealed by psychophysical experiments[11,20,21]. Positive values are shown as white and negative as black; white (black) values would then represent the activity of the corresponding ON (OFF) centre-surround ganglion cells. The patterns in *a* were first convolved with a gaussian ($\sigma = 20$ arc s) to simulate the optics of the eye; this result was then sampled at intervals of 30 arc s before convolution with the DOG operator. *c*, The result of applying the stereomatching algorithm of Marr and Poggio[16] (see legend of Fig. 3*c*) to the (binary) array shown in *b*. The grey levels here indicate the disparity of the matches obtained by the algorithm after five iterations, black corresponding to +6 pixels disparity (+3 arc min), and zero disparity appears as grey. White indicates non-matched pixels. The algorithm successfully extracts the correct disparity information without need of vernier interpolation.

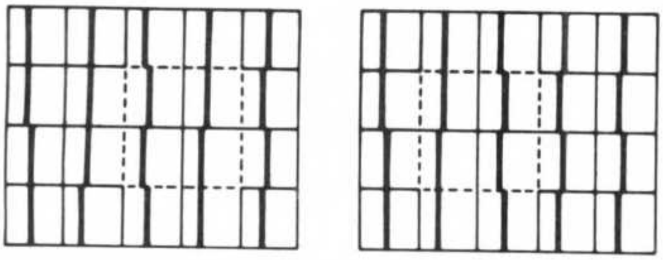

Fig. 2 Method by which the stereogram of Fig. 1*a* was generated (from Julesz and Spivack[10]).

lines; and (2) the disparity limit should be larger for horizontal random-line patterns. The regions of positive response in Fig. 1*b* are elongated slightly in the direction of the lines. This gives a larger average horizontal distance between vertical zero-crossings for the case of horizontal random-line patterns, and thus larger disparities can be viewed without confusion from false targets. Julesz and Spivack[10] report both phenomena: fusion continuing weakly with break sizes beyond the threshold for vernier acuity, and larger disparity range for horizontal patterns.

Thus, our computational experiments establish that the stereomatching problem posed by the random-line stereograms can be solved without vernier interpolation, while the psychophysical data suggest that human perception of these

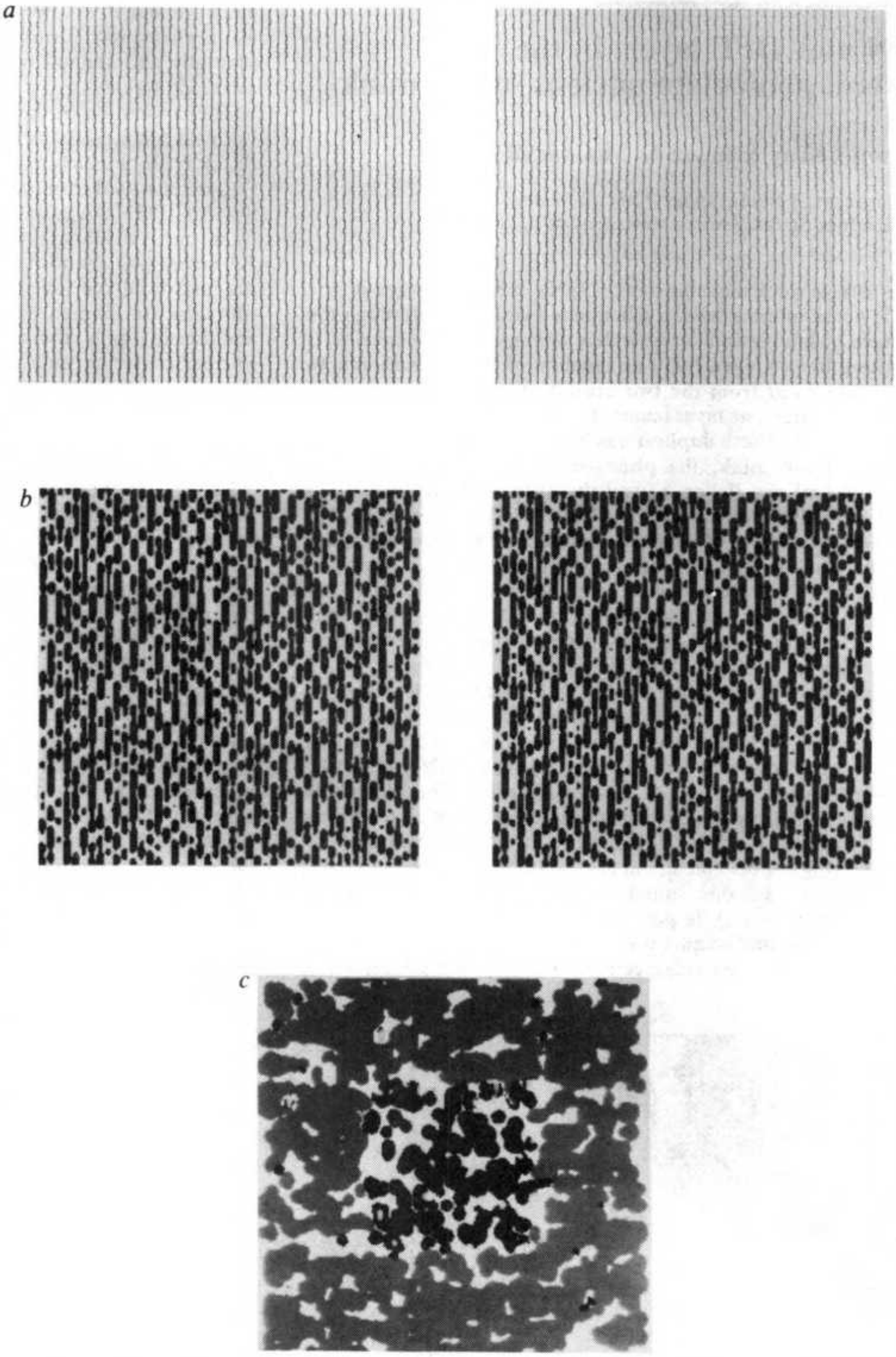

Fig. 3 *a*, A random-line stereogram with the same break size as in Fig. 1*a* but twice the line spacing. The disparity of the central square is the same as in fig. 1. *b*, The sign of the convolution of *a* with a centre-surround operator as in Fig. 1*b*. The vertical line grid in *a* dominates the coarse structure shown here; there is much less effect from the variation in line spacing due to the pattern of breaks. *c*, The result of applying the stereomatching algorithm to the array of 3*b* after five iterations. No match is obtained (white regions) over a larger portion of the array compared with Fig. 1*c*. The algorithm used here, as in Fig. 1*c*, is the cooperative algorithm of Marr and Poggio[16,17] operating on the sign of the stereo images after convolution with a DOG mask. Parameters are the same as described in ref. 16. The network corresponding to the algorithm is loaded by an 'and' operation on the 'binarized' convolved images. In this way the cooperative algorithm originally described for random-dot stereograms can be successfully used on stereo pairs of natural images, at several different resolutions (set by the size of the DOG mask). In Fig. 1*c* and 3*c* we have used seven disparity layers covering a total disparity range of ±7 pixels. We have run the algorithm with different disparity ranges and also with rather different parameters, obtaining similar results.

stereograms does not rely on vernier acuity cues. The reason for this is that the elegant random-dot and random-line stereograms of Julesz[18] and Julesz and Spivack[10], when appropriately processed, exhibit otherwise hidden cues that simplify the stereomatching problem. (The same cues may have a significant role in human stereo vision of natural images.) The stereograms therefore cannot be used to support the hypothesis that the detection of vernier breaks is computed in parallel and made available to other later processes.

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ON and OFF layers in the lateral geniculate nucleus of the mink

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The lateral geniculate nucleus (LGN), a major target of the optic nerve in mammals, is a laminated structure, although the pattern of lamination varies widely among different species. It has long been known that one function of the lamination is to segregate input from the two eyes. Many animals, however, have more than one layer innervated by each eye; the functional significance of these duplications has been only partially elucidated. In the mink, the principal layers innervated by the contralateral eye (layer A) and the ipsilateral eye (layer A1) are each split into two adjacent leaflets[1]. We report here that the anterior leaflet of each pair contains ON-centre neurones and the posterior leaflets OFF-centre neurones.

Extracellular recordings were made from the LGN of six adult male minks (*Mustela vison*) of the brown-eyed pastel variety. The minks were anaesthetized with pentobarbital, paralysed with galamine triethiodide and mechanically respirated. The corneas were protected either with 84-D contact lenses or with silicone fluid. Standard electrophysiological techniques were used[2]. Tungsten electrodes (tip impedance 10 MΩ at 1 kHz) were lowered vertically through the LGN, and visual responses were assessed at 50-μm intervals. The electrodes permitted the isolation of single units (at ~40% of all recording sites) and also recorded rich multi-unit activity. Stimuli consisted of light spots and annuli for the study of centre-surround organization. In one animal, counterphased square wave gratings (0.5 or 1 cycle per deg) were also used to assess the linearity of spatial summation within receptive fields. Points of interest were marked with electrolytic lesions (3–5 μA for 3–5 s, electrode negative). At the end of the experiment, the animal was perfused with formol–saline. The LGN was sectioned parasagittally and stained with cresyl violet for reconstruction of electrode tracks.

The lamination of the mink LGN is illustrated in Fig. 1*a*, *b*. Its most striking feature is the pair of A laminae (A_{ant} and A_{post}), both of which receive input from the contralateral eye[1]. A_{ant} is separated from the optic radiation by the perigeniculate nucleus (PGN). A_{ant} and A_{post} are separated from each other by a thin cell-sparse zone. Behind the A laminae lie the two A1 layers ($A1_{ant}$ and $A1_{post}$), both of which are innervated by the ipsilateral eye. The A1 laminae differ from the A layers in that they are thinner, less clearly separated from one another, and occupy only the ventromedial portion of the nucleus. Further posterior are a thin magnocellular layer innervated by the contralateral eye (C_m), and at least two parvocellular C layers (C_p). Aside from the sublamination of the A and A1 layers and the large size of the monocular segment, the mink LGN resembles that of the cat[3].

Because of the curvature of the layers, an electrode driven vertically through the LGN will pass obliquely or tangentially through several or all of the layers. One such penetration is illustrated in Fig. 1*a*, *b*. In the C layers, mixed ON- and OFF-centre responses were obtained on stimulation of the contralateral eye. In the A sublaminae, cells were also driven by the contralateral eye, but ON-centre responses were confined to A_{ant} and OFF-centre to A_{post}. The transitions between ON- and OFF-centre responses were quite abrupt: one point of transition from ON- to OFF-centre activity was marked by a lesion (L1 in Fig. 1*b*). The lesion was centred on the cell-sparse zone between A_{ant} and A_{post}. A similar functional pattern was revealed in the A1 sublaminae, with the difference that the cells were driven by the ipsilateral eye. Again, ON-centre activity was confined to the anterior leaflet of the pair and OFF-centre responses to the posterior leaflet. A lesion was placed at the ON/OFF transition point (L2 in Fig. 1*b*). Histologically, the subdivision of layer A1 was not as distinct as that of layer A; nevertheless, the lesion lay squarely in the middle of the layer.

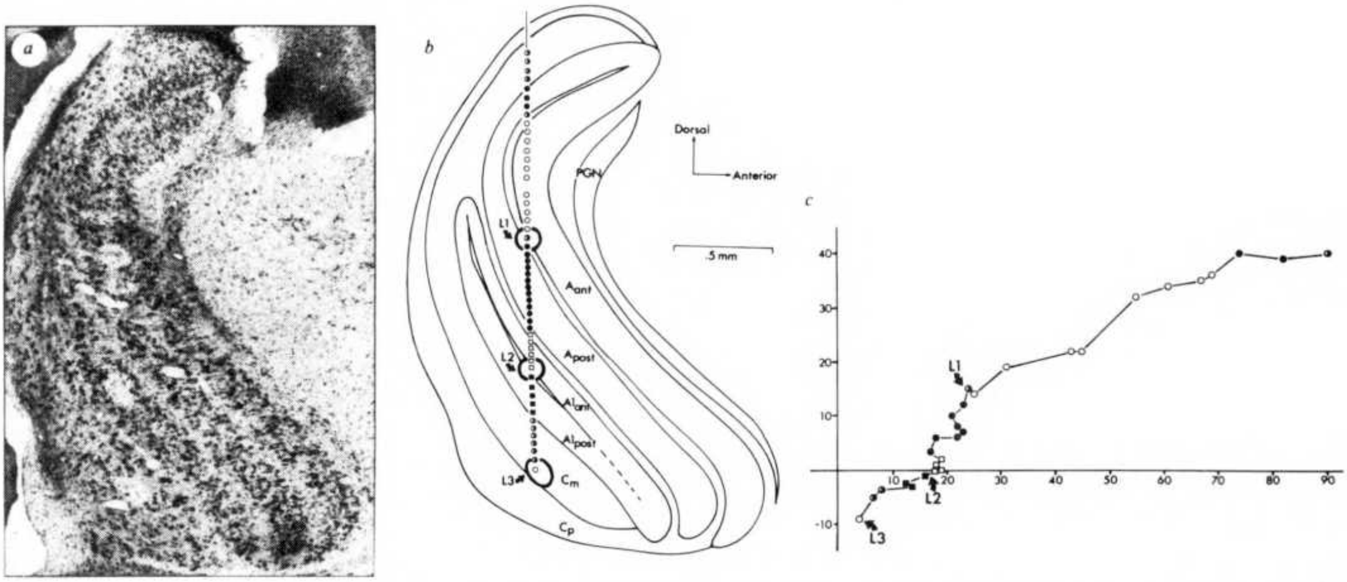

Fig. 1 Reconstruction of an electrode penetration through the left LGN of a mink. *a*, Parasagittal section (anterior to the right) stained with cresyl violet. Three electrolytic lesions made during the penetration are visible as pale spots. *b*, Key to *a*, showing the outlines of the layers, the positions of the three lesions (L1, L2, L3) and the responses obtained at 50-μm intervals along the track (intervals were corrected for differential shrinkage). Circles indicate responses to the contralateral eye and squares to the ipsilateral eye. Open symbols indicate ON-centre responses, filled symbols indicate OFF-centre responses, and half-filled symbols indicate mixed responses. The first two lesions (L1 and L2) mark transitions from ON-centre to OFF-centre responses, and lie between the anterior and posterior leaflets of layers A and A1 respectively. The third lesion (L3) marks the end of the penetration. *c*, Progression of receptive fields across the contralateral (right) visual field, for the penetration illustrated in *a*, *b*. The axes represent the vertical and horizontal meridians. Symbols as in *b*. Each symbol is placed at the centre of the unitary or multi-unit activity recorded at a single site. Only those recording sites that gave distinct shifts in receptive field position are indicated. Fields moved downwards and medially during the penetration.

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Table 1 Laminar distribution of ON- and OFF-centre responses in the mink LGN

	A_{ant}	A_{post}	$A1_{ant}$	$A1_{post}$	C
ON-centre	107 (100%)	0	23 (96%)	1 (7%)	24 (38%)
OFF-centre	0	62 (97%)	1 (4%)	14 (93%)	9 (14%)
Mixed	0	2 (3%)	0	0	30 (48%)

Responses at 210 recording sites in 14 penetrations are pooled The electrodes were advanced in 50-µm steps; a step was counted as a recording site if· (1) there was a distinct shift in receptive field position from the previous site, and (2) the laminar location of the site was subsequently determined histologically The anterior leaflets of layers A and A1 contained predominantly ON-centre responses, the posterior leaflets OFF-centre responses, and the C layers mixed responses.

Results obtained from a further 13 penetrations in 6 animals corroborated this pattern of organization (Table 1). ON-centre responses (both single unit and multi-unit activity) were found in the anterior leaflets of A and A1 and OFF-centre responses in the posterior leaflets. This was true for both the binocular and monocular portions of the LGN. Exceptions to this pattern were found at only 4 recording sites out of a total of 210 sites in these layers. Eight ON/OFF transitions were marked with lesions; in each case, the lesion lay between the anterior and posterior leaflets of A or A1. In the C layers, on the other hand, mixed responses predominated, although we twice encountered sequences of pure ON-centre responses and must therefore leave open the possibility of some functional segregation.

Because a vertical penetration through the LGN passed roughly parallel to the layers, receptive field positions traversed a wide extent of the visual field map (Fig. 1*c*). Starting at the far periphery of the contralateral field at a high elevation, fields progressed downwards and medially, and ended near the vertical meridian in the lower quadrant. We have not examined the layout of the visual field map in detail, but it seems to resemble that seen in the posterior upturned portion of the cat LGN[4].

Aside from the segregation of ON- and OFF-centre responses, receptive field properties of cells in the mink LGN also resembled those seen in the cat[5,6]. Most cells in the A and A1 layers exhibited a typical centre-surround organization. They responded optimally to the onset or offset of a small light spot (1–2.5 deg diameter); increasing the spot size diminished the response but never reversed its sign. Responses of opposite sign could be obtained by stimulating the surround with annuli. No colour-coded responses were found when cells in layers A and A1 were tested with red, green or blue spots and annuli. In one animal, we tested 19 units in the A and A1 layers for linearity of spatial summation using a counterphased square wave grating. For some of these cells we could find a null position of the grating; others had no null position, but showed a frequency doubling[7]. Our findings suggest that, as in the cat[6], X- and Y-like cells are intermingled in layers A and A1. We did not examine the properties of layer C cells in detail.

Our results demonstrate that the laminar organization of the mink LGN has a clear functional significance, serving to segregate cells not only by eye preference but also on the basis of ON- or OFF-centre responses. The pattern of lamination in the LGN varies considerably among mammalian species. Available physiological evidence suggests that the layers may separate different aspects of visual information in different ways. For example, the mink and cat do not seem to segregate X- and Y-like cells sharply, but such a segregation has been reported for the monkey[8], tree shrew[9] and bush baby[10]. ON/OFF segregation has been reported for the tree shrew[9], while the macaque monkey appears to segregate ON- and OFF-centre units to some degree[11]. Among carnivores, the cat mixes ON- and OFF-centre cells within each layer, whereas recent work on the ferret (K. Zahs and M. P. Stryker, personal communication), a close relative of the mink, suggests an organization similar to that found in the present study.

One function of laminar specialization in the LGN may be to preserve the separation of functional channels up to the cortical level. Segregation of ON- and OFF-centre channels is already evident in the stratification of ganglion cell dendrites in the inner plexiform layer of the retina[12]. Because it is thought that ON- and OFF-centre geniculate neurones are driven by retinal ganglion cells of the same sign[5], it seems likely that axon terminals of ganglion cells terminate selectively in the appropriate leaflets of layers A or A1 in the mink LGN. Anatomical studies in several species have shown that individual layers of the LGN may project differentially to visual cortex: their terminals may occupy separate columns or layers[13,14]. It would be interesting to examine whether the ON and OFF sublaminae of the mink LGN also project differentially to visual cortex.

Another possibility is that laminar segregation permits separate modulation of different channels of visual information. Transmission of information through the LGN is under the influence of reticular, corticofugal and intrageniculate systems[15,16]. The segregation of ON- and OFF-centre channels into separate layers may facilitate their independent modulation, but the advantage to the mink of such an arrangement is unclear.

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Na^+–K^+–Cl^- co-transport in the intestine of a marine teleost

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A key element in the absorption of salt and water by various epithelia is the presence, in the apical or brush border membrane, of a mechanism for directly coupling the inward movements of Na^+ and Cl^- (ref. 1). In this way the downhill gradient for Na^+ from lumen to cell, which is maintained by the cell's Na^+/K^+ pump, can provide the energy for cellular accumulation and transepithelial transport of Cl^-. A requirement for K^+ at the serosal surface of the epithelium has long been accepted as essential to the operation of the Na^+/K^+ pump and therefore to salt absorption. Until very recently, however, the effect of luminal K^+ on NaCl absorption has not been explored. We show here that luminal K^+ stimulates NaCl absorption in the intestine of a marine teleost, the winter flounder,

***Pseudopleuronectes americanus.* Furthermore, K^+ uptake across the brush border membrane depends on the presence of both Na^+ and Cl^- in the bathing medium and is inhibited by furosemide, which also inhibits the coupled uptake of Na^+ and Cl^- (ref. 2). Thus, flounder intestine seems to have a Na^+–K^+–Cl^- co-transport system similar to that demonstrated in erythrocytes[3–5], cultured tumour cells[6], cultured kidney cells[7] and squid giant axon[8]. Electrophysiological studies of the thick ascending limb of Henlé's loop in mammalian kidney[9] and the diluting segment in amphibian kidney[10] also indicate that luminal K^+ is required for salt absorption. Co-transport of Na^+, K^+ and Cl^- may therefore be characteristic of a variety of cells and tissues, including salt-absorbing epithelia.**

Flounder intestine is morphologically less heterogeneous than the intestines of higher vertebrates; in particular, it contains no crypts[11]. (Possibly for this reason, it cannot be stimulated to secrete NaCl and water[12], differing in this respect from avian and mammalian intestine, both of which possess crypts.) In short-circuit conditions and in normal Ringer's solution containing 5 mM K^+, flounder intestine absorbs both Na^+ and Cl^- (ref. 11); neither is absorbed, however, in the absence of the other[11]. The site of direct coupling between Na^+ and Cl^- movements is the brush border membrane, and the coupling ratio is close to 1 : 1 (ref. 2).

We have examined the effect of a K^+-free luminal medium on Cl^- fluxes and the effects of ion substitutions, furosemide, bumetanide and Ba^{2+} on both K^+ fluxes and the electrical potential difference across the apical membrane (Ψ_a). Unidirectional and net transepithelial ion fluxes and initial rates of uptake from the luminal solution into the epithelium (unidirectional influxes) were measured using radioisotopes as described previously[2,11]. Conventional voltage-sensing microelectrodes were used to determine Ψ_a (ref. 13). K^+ transport was evaluated by measuring ^{86}Rb fluxes in tissues preincubated in K^+-free, Rb-containing Ringer's. Preliminary studies indicated that transepithelial fluxes of 5 mM ^{42}K and ^{86}Rb across flounder intestine are identical and that the transepithelial potential difference (Ψ_t), short-circuit current (I_{sc}) and Ψ_a are equally well maintained whether Rb-Ringer's or K^+-Ringer's is used[14,15].

Table 1 shows the effects of Ba^{2+} and furosemide on transepithelial Rb fluxes. In their absence, short-circuited flounder intestine secretes Rb; This seems to be due to active uptake across the basolateral membrane by the Na^+–K^+ pump and diffusional exit across the apical membrane[13]. When Ba^{2+} is added to the luminal bathing medium, the direction of Rb transport reverses from secretion to absorption. Consistent with its effect on K^+ channels in several epithelia[16–18], Ba^{2+} blocks an apical membrane K^+ channel in flounder intestine[14,15]. When furosemide is then added to the luminal bathing medium, the Ba^{2+}-stimulated Rb absorption ceases.

Table 2 demonstrates that this inhibitory action of furosemide is exerted on a K^+ uptake process in the brush border membrane. Other characteristics of this process are also shown in Table 2: (1) Rb influx is reduced by 80% by furosemide so that, at most, 20% is paracellular. (2) Cl^- removal reduces Rb influx to the same level as does furosemide. (3) Na^+ removal reduces Rb influx to the same level as does Cl^- removal. (4) Rb influx is 6–10 times greater than the unidirectional transepithelial Rb flux from mucosa→serosa, suggesting that K^+ transport across the basolateral membrane is normally rate-limiting in transepithelial K^+ absorption. (5) Ba^{2+}, despite its profound effect on transepithelial Rb fluxes, has no discernible effect on Rb influx. This surprising observation suggests that ^{86}Rb entry into the cell via the apical membrane K^+ channel is normally small. This channel, therefore, seems to strongly rectify Rb movements. Similar rectification of K^+ transport has been demonstrated in turtle colon[19] and in excitable tissues[20].

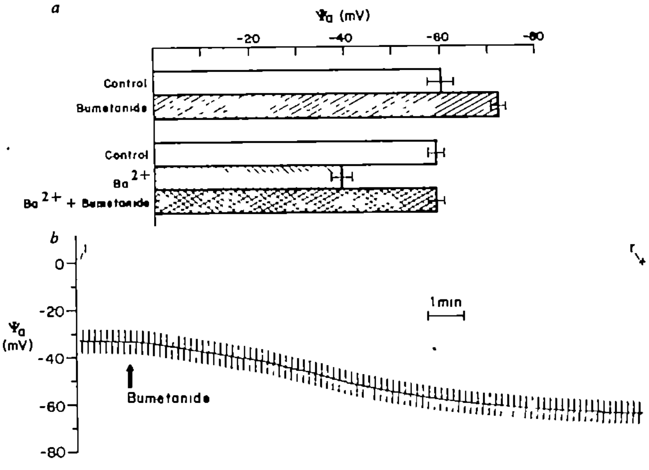

Fig. 1 Effect of bumetanide on the electrical potential difference across the apical membrane (Ψ_a). *a*, Bumetanide (10^{-4} M) was added to the mucosal solution in the presence ($n = 14$) or absence ($n = 8$) of mucosal Ba^{2+} (2.0 mM). Brackets indicate ±1 s.e. Note the depolarization of Ψ_a by Ba^{2+}, due to blockage of apical K^+ conductance, and hyperpolarization of Ψ_a by bumetanide in the presence or absence of Ba^{2+}. Similar results were obtained with Cl^--free mucosal solutions (see text). At least four separate recordings of Ψ_a were obtained in each experimental condition, for each tissue. *b*, Single recording of Ψ_a obtained in the presence of mucosal Ba^{2+} (2.0 mM) showing the hyperpolarization induced by addition of bumetanide (arrow) to the mucosal solution. i, Impalement of cell; r, electrode retracted. The deflections in Ψ_a result from passage of bipolar, constant-current pulses across the tissue for determination of fractional apical membrane resistance.

The data in Table 2 suggest that K^+ influx across the brush border is linked to the influxes of both Na^+ and Cl^-. To determine the influence of luminal K^+ on Cl^- transport, transepithelial Cl^- fluxes and Cl^- influxes were measured in the presence and absence of luminal K^+ (Table 3). Removing luminal K^+ (with serosal K^+ maintained at 5 mM) reduced net Cl^- flux by 60% (Table 3*a*) and also reduced Cl^- influx (Table 3*b*); indeed, the furosemide-sensitive portion of the Cl^- influx was reduced by 70%. Although not directly measured, Na^+ influx is almost certainly also reduced by removing K^+ from the lumen as K^+ influx requires luminal Na^+ (Table 2) and because furosemide reduces Na^+ influxes[2] as well as Cl^- and K^+ influxes. The furosemide-inhibitable Cl^- influx remaining

Table 1 Effects of luminal Ba^{2+} and furosemide on transepithelial Rb fluxes across short-circuited flounder intestinal mucosa

	J_{ms}	J_{sm}	J_{net}	I_{sc}	G
Control (9)	0.34±0.05	1.04±0.09	−0.70±0.09	−3.58±0.22	26.5±1.1
Ba^{2+} (9)	1.09±0.10	0.48±0.10	+0.63±0.09	−0.82±0.26	21.2±1.8
Ba^{2+}+furosemide (4)	0.50±0.02	0.48±0.06	+0.02±0.08	−0.21±0.38	23.8±2.2

Values shown are mean±s.e. for (n) experiments. J_{ms} and J_{sm} are unidirectional fluxes from mucosa (m)→serosa (s) and s→m respectively; $J_{net} = J_{ms} - J_{sm}$. Rb fluxes and short-circuit current (I_{sc}) are in μequiv. h^{-1} cm^{-2} and conductances (G) in mS cm^{-2}. Intestine stripped of muscularis and mounted in chambers was bathed in K^+-free, Rb-containing Ringer's. The medium was buffered to pH 8.0 with 5 mM N-2-hydroxyethyl piperazine sulphonic acid and also contained (in mM): NaCl, 170; $RbNO_3$, 5; $CaCl_2$, 1.25; $MgCl_2$, 1.1; Na_2HPO_4, 3; D-glucose, 20. It was maintained at 15 °C and continuously bubbled with room air. Flux measurements were begun 45–60 min after adding ^{86}Rb and 15 min after adding Ba^{2+} (to 5 mM) or furosemide (to 0.4 mM) to the luminal side.

Table 2 Effects of furosemide, Ba^{2+} and Cl^- and Na^+ replacements on lumen-to-epithelium influx of Rb

	Rb influx, μequiv. h^{-1} cm^{-2}	
	−Furosemide	+Furosemide
Effect of Ba^{2+}(4)		
Control	2.13±0.32	0.48±0.16
Ba^{2+}	2.39±0.27	0.38±0.06
Effect of Cl^- replacement (4)		
Control	3.30±0.18	0.70±0.12
Cl^--free	0.64±0.09	0.71±0.15
Effect of Na^+ replacement (8)		
Control	2.80±0.24	–
Na^+-free	0.69±0.12	–

Values shown are ± 1 s.e. for (n) experiments. Control tissues were preincubated in the Rb-containing Ringer's solution described in Table 1. Where indicated, Cl^- was replaced by gluconate and Na^+ by either N-methyl-D-glucamine or tetraethylammonium. After 60 min, ^{86}Rb was added and its influx determined over 90 s (Rb uptake is linear with time for at least 120 s). Tissues were short-circulated during the influx measurements The influx technique is described in detail elsewhere[2]. Ba^{2+} (to 5 mM) and furosemide (to 0.4 mM) were added to the luminal side 15–20 min before adding ^{86}Rb.

after K^+ removal from the luminal bathing medium may result from the persistence of small amounts of K^+ in the unstirred layer at the brush border surface, as the enterocytes normally secrete K^+.

The coupling stoichiometry of the brush border Na^+–K^+–Cl^- co-transport process remains to be accurately determined. Tables 2 and 3b suggest more than one Cl^- per K^+. Our previous determinations of furosemide-inhibitable Na^+ and Cl^- influxes yield a coupling ratio (±1 s.e.) of 1.3±0.4 Na^+ per Cl^-, which does not differ significantly from 1 : 1 (ref. 2). These estimates of transport stoichiometry, although admittedly inexact, suggest that in flounder intestine the brush border co-transport process is electrogenic, translocating more cations than anions.

Transport-related changes in Ψ_a are consistent with these kinetic data. Inhibition of transport by addition of the furosemide analogue, bumetanide, hyperpolarized Ψ_a by 12 ± 2 mV (Fig. 1a). Similarly, complete replacement of luminal Cl^- with gluconate hyperpolarized Ψ_a by 14 ± 5 mV ($n = 3$). Cl^- replacement would be expected to depolarize Ψ_a if significant Cl^- conductance were present in the apical membrane. Finally, complete replacement of luminal Na^+ with N-methyl-D-glucamine hyperpolarized Ψ_a by 15 mV (mean of two experiments). All these changes are consistent with inhibition of a positively charged co-transporter. Similar hyperpolarizations have been reported for two other epithelia exhibiting K^+-dependent NaCl co-transport: the thick ascending limb of Henle's loop in mammalian kidney[9] and the diluting segment in amphibian kidney[10].

Table 3 Dependence of Cl^- fluxes on luminal K^+

a Transepithelial fluxes (9)

$[K^+]_m$	J_{ms}	J_{sm}	J_{net}	I_{sc}
5 mM	6.17±0.48	2.63±0.33	3.54±0.32	−1.78±0.16
0	3.93±0.62*	2.49±0.44	1.44±0.56*	−0.93±0.10*

b Influxes (9)

$[K^+]_m$	−Furosemide	+Furosemide	Δ
5.mM	15.83±2.09	8.20±1.75	7.63±0.80
0	12.24±1.29†	9.97±1.42	2.27±0.56†

Values shown are mean μequiv h^{-1} cm^{-2} ± 1 s.e. for (n) experiments. The medium was the same as that described in Table 1 except that KCl replaced $RbNO_3$. Serosal K^+ concentration was always 5 mM. $[K^+]_m$, luminal K^+ concentration. The net Cl^- flux in a is about one-third less than that usually measured [2,11,12]. Cl^- influx was measured with ^{36}Cl over 35 s. Furosemide was added (to 0.4 mM) to the luminal side 15 min before influx measurements. See Tables 1 and 2 for further details. Less than control: * $P < 0.05$; † $P < 0.01$.

The hyperpolarization of Ψ_a observed with inhibition of co-transport was even larger in the presence of Ba^{2+}. Addition of Ba^{2+} to the luminal solution depolarized Ψ_a (Figure 1a) and increased the fractional apical membrane resistance ($\Delta\Psi_a/\Delta\Psi_t$ in response to constant current pulses) from 0.19±0.02 to 0.61±0.03 ($P < 0.01$; $n = 25$). This is consistent with the inhibitory effect of Ba^{2+} on apical membrane K^+ conductance[14]. In the presence of Ba^{2+}, Ψ_a hyperpolarized by 20±2 mV when bumetanide was added (Fig. 1a) and by 21±4 mV when Cl^- was replaced by gluconate ($n = 4$). Figure 1b shows the time course ($t_{1/2} = 5$ min) of the bumetanide-induced hyperpolarization. The time required for bumetanide to block the brush border co-transporter is unknown. Since the apical K^+ conductance channel is parallel to the co-transport pathway, Ba^{2+} would be expected to magnify the effect on Ψ_a of blocking an electrogenic co-transporter. The hyperpolarization of Ψ_a was probably not due to changes in cellular K^+ or Cl^- activities that might have resulted from inhibition of co-transport as hyperpolarization persisted when apical membrane K^+ conductance was blocked by Ba^{2+} and because significant basolateral membrane K^+ or Cl^- conductances have not been detected in ion replacement studies[14]. However, the possibility that there is an obligatory inverse relationship between apical membrane K^+ conductance and co-transport rate cannot be excluded. Additional studies are needed to define whether the epithelial co-transport process (in contrast to that of avian erythrocytes[5]) is affected by changes in membrane potential.

The results presented here suggest that what was initially conceived to be an epithelial membrane Na^+–Cl^- co-transport is in fact a Na^+–K^+–Cl^- co-transport; the involvement of this triple co-transport in cell homeostasis is largely unexplored. It may facilitate regulation of cell volume in non-epithelial cells and regulation of fluid transport across low resistance epithelia. As long as the basolateral membrane Na^+–K^+ pump operates normally, Na^+ and Cl^- activities in the epithelial cell are less than their extracellular activities. In an electrically neutral co-transport process involving only Na^+ and Cl^-, entry is in the direction of the chemical potential differences for both ions and net transport could only be regulated by modifying co-transport capacity through the action of intracellular mediators such as Ca^{2+} or cyclic nucleotides. If K^+ also participates in the co-transport process, however, the outwardly directed K^+ gradient could, in principle, assist in regulation of net Na^+ and Cl^- entry. Conditions that might alter net flux through the co-transport pathway, including its voltage-dependence and affinities for the transported ions, remain to be defined.

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Oocyte extracts reactivate developmentally inert *Xenopus* 5S genes in somatic nuclei

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In *Xenopus* and other amphibians, there are two major classes of 5S RNA gene[1]. One class, which codes for somatic-type 5S RNA, is expressed in both oocytes and somatic cells[2]; the other, which is 50 times more abundant[3] and which codes for oocyte-type 5S RNA, is expressed in oocytes but not in somatic cells[2,4]. The developmentally inactivated oocyte-type genes are reactivated when erythrocyte nuclei are injected into most oocytes (activating oocytes)[5]. As the injected nuclei are induced to take on the natural activity of the host cell, the activation of oocyte-type 5S genes may represent a normal control mechanism. A special opportunity to investigate this mechanism is provided by the existence of certain individual animals whose oocytes consistently fail to activate 5S genes (non-activating oocytes). We have injected extracts of activating or non-activating oocytes, together with erythrocyte nuclei, into oocytes of each kind. We show here that the transcription of 5S genes is induced by extracts of activating oocytes (positive regulation) and is not inhibited by similar extracts of non-activating oocytes. The activating factor is sensitive to protease and heat treatment, suggesting that a protein is involved.

The procedures for preparing oocyte extracts and somatic nuclei from erythrocytes[5,6] are given in Fig. 1 legend. Somatic nuclei from mature erythrocytes were injected, either alone or after mixing with extracts prepared from either activating or non-activating oocytes, into the germinal vesicles of both types of oocyte. The oocytes were then injected with 1 μCi [α-^{32}P]GTP and incubated at 19 °C for 36 h[5]. Labelled transcripts were extracted and electrophoresed on non-denaturing 17% polyacrylamide gels to differentiate between somatic- and oocyte-type 5S RNA[5].

The results are shown in Fig. 1. As found previously[5], the oocyte 5S genes are transcribed in nuclei injected into activating oocytes (Fig. 1, lanes 3–5), but not in those injected into non-activating oocytes (lanes 1 and 2). When somatic nuclei were mixed with extracts of activating oocytes and injected into non-activating oocytes, the previously inactive oocyte-type 5S RNA genes in the somatic nuclei were transcribed about as efficiently as when such nuclei were injected into activating oocytes (Fig. 1, lanes 6 and 9). Thus, activating extracts have a strongly positive effect. On the other hand, extracts from non-activating oocytes injected with somatic nuclei into activating oocytes did not diminish the expression of the oocyte-type 5S RNA genes (Fig. 1, lanes 14 and 15). In addition, activating extracts mixed with non-activating extracts in a 1:1 ratio and injected with somatic nuclei into non-activating oocytes, continue to induce efficient transcription of oocyte-type 5S genes (data not shown). Thus, non-activating oocytes or extracts prepared from these oocytes do not contain inhibitors or nucleases specific for the oocyte-type 5S genes or their products. Extracts from activating or non-activating oocytes had no effect when injected into the same kind of oocyte (Fig. 1, lanes 10–13). In all injected oocytes, the somatic-type 5S genes of the somatic nuclei were transcribed efficiently (see Fig. 1); this serves as an internal control of the viability of the oocytes and their ability to transcribe the injected 5S genes. As activating extracts exert their influence in non-activating oocytes and not vice versa, it seems that at least one factor controlling the reactivation of oocyte 5S genes regulates 5S transcription in a positive fashion.

We have analysed the activating extract by incubating it at 70 °C for 3 min, or by incubating it with the protease papain. Somatic nuclei were mixed with the heated extract and injected into oocytes, as described above, and RNA was extracted and analysed (Fig. 1, lane 16). The oocyte 5S genes were not activated, indicating that at least one component of the activating factor is heat-labile. Activating extracts were also protease-treated by incubating with papain and neutralizing with antipain as described in Fig. 2 legend, before being mixed with somatic nuclei and injected into the germinal vesicles of non-activating oocytes. Activating potential was destroyed by the protease (Fig. 2, compare lanes 12–14 with lanes 9–11). However, when untreated activating extract was added back to the papain-antipain-treated extract, and injected as above, the oocyte-type 5S genes were transcribed (Fig. 2, lanes 15–17). Somatic nuclei mixed with activating extracts that had been pre-incubated in papain digestion buffer alone were also injected into non-activating oocytes as a further control for 5S transcription. In these conditions, the oocyte-type 5S RNA genes are transcribed (Fig. 2, lanes 18–20).

As the activating factor is heat-labile and protease-sensitive, it probably consists of one or more proteins, which act positively to activate 5S transcription. It has previously been shown that

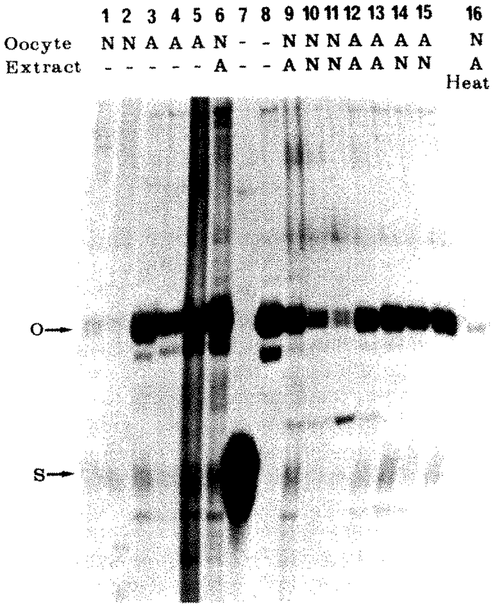
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Oocyte N N A A A N - - N N N A A A A N
Extract - - - - - A - - A N N A A N N A
Heat
O
S

Fig. 1 Transcription of 5S RNA genes with activating and non-activating oocyte extracts. The extracts were prepeared as follows: 200 single oocytes were isolated free of connective tissue, washed twice in distilled water, all excess fluid removed, then gently homogenized in a glass–glass dounce homogenizer. The extract was then centrifuged in an Eppendorf microfuge at 10,000*g* for 10 min at 4 °C, the supernatant was re-centrifuged at 10,000*g* for 15 min and the supernatant used. Somatic nuclei were prepared by lysolecithin treatment of mature erythrocytes[6]. Extracts were diluted 1:1 with somatic nuclei suspended in injection medium, just before injection into oocytes. Oocytes were injected, aiming for the germinal vesicle, with the relevant extract. After 4 h, 1 μCi [α-^{32}P]GTP was injected into the cytoplasm of each oocyte. After incubating at 19 °C for 36 h, labelled transcripts were extracted and run on a non-denaturing 17% polyacrylamide gel[5]. Each lane contains half the RNA from one oocyte. All the oocytes shown received an injection of erythrocyte nuclei. For each lane, the type of oocyte used and the source of extract injected (if any) is indicated: N, A, oocytes or extracts from non-activating or activating females, respectively. In lane 16, the activating extract was heated at 70 °C for 3 min. Lanes 7 and 8 are markers showing somatic- and oocyte-type 5S RNA, respectively (O and S at the left of the figure). The slight increase in oocyte-type 5S RNA in lanes 10 and 11 relative to lanes 1 and 2 is not always observed, and is small compared to the increase in lanes 6 and 9. It is enhanced by the long autoradiographic exposure used in these experiments.

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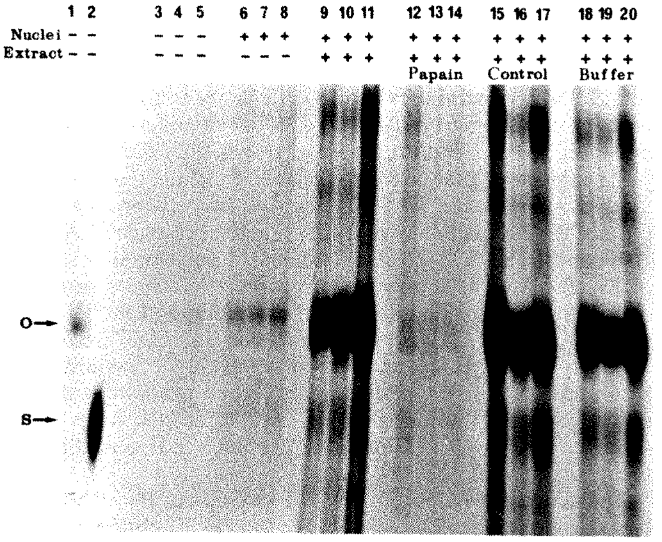

Fig. 2 Transcription of 5S RNA genes injected with activating extracts treated with protease. Oocyte extracts and somatic nuclei were prepared as described in Fig. 1 legend. All injections were into oocytes of non-activating females. Oocytes were injected with the samples and 4 h later with 1 μCi[α-^{32}P]GTP. Labelled transcripts were extracted and analysed[5]. Lanes 1 and 2 contain marker 5S RNAs (O, oocyte-type; S, somatic-type). Lanes 3–20 were either injected (+) or not injected (−), with erythrocyte nuclei and/or with an extract of activating oocytes, as indicated. Lanes 3–5 represent endogenous transcription. Lanes 12–14, injection of somatic nuclei mixed with activating extract, which had been pre-incubated with 0.8 mg ml^{-1} papain (Sigma) at 20 °C for 1 h, followed by neutralization with 1.25 mg ml^{-1} antipain (Sigma) for 10 min at 20 °C. Lanes 15–17, injection of somatic nuclei mixed with a 1:1 ratio of papain–antipain-treated extracts (as in lanes 12–14) and untreated activating extracts. Lanes 18–20, injection of somatic nuclei mixed with activating extract and papain buffer (without papain) which had been incubated together at 20 °C for 1 h.

at least two proteins in addition to RNA polymerase III are required for 5S RNA transcription[7,8]. Only one of these, transcription factor IIIA (TFIIIA), has been purified[7]. However, TFIIIA alone does not account for the activity in activating extracts since injection of somatic nuclei plus purified TFIIIA into non-activating oocytes is not sufficient to reactivate the oocyte 5S genes[5]. Whether the activating factor acts independently of, or in conjunction with, TFIIIA has yet to be elucidated. The factor functions in the absence of DNA replication, since the mature erythrocytes used as a source of somatic nuclei represent a terminal cell type in which no DNA replication occurs[9] and oocytes do not induce DNA synthesis in injected nuclei[10].

The activating and non-activating oocytes used in these experiments represent the extremes of a continuous range of activating potential. All oocytes have sufficient activating factor to ensure the expression of their own oocyte-type 5S genes (unpublished result). However, it seems that only some have an excess sufficient to activate the extra 5S genes from several hundred injected somatic nuclei. The fact that all oocytes of a given female maintain indefinitely the same activating or non-activating property has allowed us to make use of this variation for the experiments described.

The regulation of gene expression, on the molecular level, is not understood for any eukaryotic gene. We have shown for a particular gene system, the oocyte-type 5S RNA genes, that specific, although so far unidentified, protein factors can regulate transcription in a positive fashion. We are now attempting to purify these factors.

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T-cell hybridomas which produce B lymphocyte replication factors only

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B lymphocytes stimulated with BRMF (B-cell replication and maturation factor) expand clonally and mature into immunoglobulin (Ig)-secreting cells. BRMF may be a single or separate factors (BRF, BMF), capable of inducing replication and maturation in B cells[1–3]. Experimental evidence from others[4,5] suggests that BRF and BMF may be distinct molecular entities. We now find that some hybrids of concanavalin A (Con A)-activated splenic T cells and the thymoma BW5147 produce BRF on stimulation with Con A, but do not produce BMF, T-cell growth factor (TCGF), T-cell replacing factor (TRF) or factors stimulating myeloid or erythroid colonies (CSF). Thus BRF and BMF are distinct activities. BRF produced by the T-cell hybridoma A32-26 appears in two major forms with molecular weights of 15,000–20,000 and 80,000–90,000.

BRMFs are made in interactions of helper T cells, Ia-compatible accessory cells and antigen[6–17]. In the absence of antigen and helper T cells, resting B cells respond to BRMF by polyclonal maturation without replication[3]. B-cell maturation-inducing activity can be quantitated in plaque assays as the number of immunoglobulin-secreting cells (plaque-forming colonies, PFC)[18]. In order to replicate in response to BRMF, resting B cells must be raised[3,19] to the 'excited' state, either by concomitant binding of antigen and interaction of helper T cells with Ia (antigen-specific, Ia-restricted, T-dependent activation) or by mitogens, such as lipopolysaccharide (LPS)[20], which circumvent binding to immunoglobulin and Ia (polyclonal, Ia-unrestricted, T-independent activation)[8]. Restimulation of LPS-activated B-blasts has provided a convenient assay for replication-stimulating activity[1,21]. All previous BRMF preparations, as well as LPS, have also been found to induce further maturation to immunoglobulin-secreting PFC.

Cloned, antigen-specific helper T cells, as well as T-cell hybridomas, have been shown to produce several factors, including T-cell growth factor (TCGF, IL-2), BRMF, TRF and factors stimulating erythroid and myeloid colony formation (CSF) (refs 22, 23). Many of these lines have been found to produce more than one of these factors at the same time.

In an attempt to find T-cell hybridomas producing only one of these activities, we have fused BW5147 thymoma cells with activated spleen cells. (The thymoma BW5147 alone does not produce any of the activities tested.) Of 150 cloned hybridomas obtained in one fusion, 15 produced considerable quantities of factors when stimulated with Con A. Six produced TCGF, BRMF and CSF, and three produced TCGF and BRMF and no CSF. Two T-hybridoma produced only CSF and four others produced only BRMF. One of the T-cell hybridomas obtained in a similar fusion by Köhler *et al.*[24], called A32-26, produced also only BRMF (Table 1). This hybridoma which had already been recloned and which produced the highest levels of BRMF, was selected for further studies.

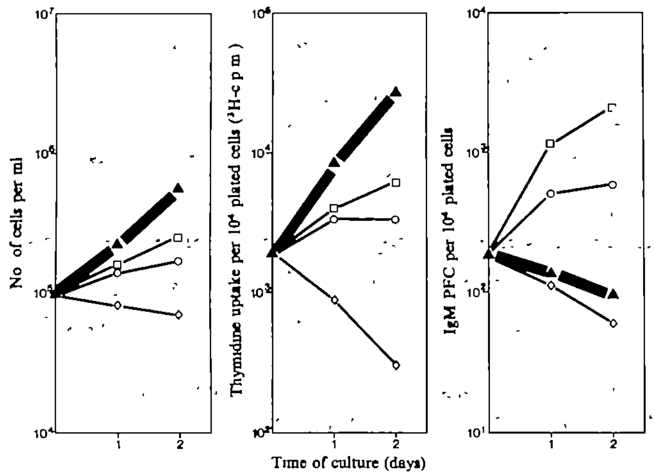

Fig. 1 Replication of LPS-activated B cell blasts (§, Table 1) in the presence of factors produced by Con A-stimulated A32-26 T hybridoma cells (*, Table 1) (—▲—), of factors produced by Con A-stimulated rat spleen cells (†, Table 1) (—□—), of LPS (50 μg ml^{-1}) (—○—), or in the absence of any added factors (—◇—). Replication was measured either by cell counts (left) or by uptake of radioactive thymidine (‡ and §, Table 1) (centre). Maturation was measured as the increase in the number of IgM PFC in culture (right). IgG- and IgA-secreting PFC (not shown in the figure) behaved as the IgM PFC.

First we investigated whether Con A-stimulated A32-26 T hybridoma cells released both replication-stimulating and maturation-inducing factors. For controls the mitogen LPS and factors produced by the interaction of cloned helper cells, antigen and histocompatible macrophages[25] were used. LPS-activated B-blasts could be restimulated to continued replication by A32-26 factors, LPS or factors of T-cell help, as measured either by cell counts or by the uptake of radioactive thymidine (Fig. 1). LPS and factors of T-cell help increased the number of IgM-secreting PFC but factors from A32-26 T hybridoma did not. Replication but no increased maturation was observed over a 300-fold range of concentrations of A32-26 factors of 50–0.15% in culture. In control experiments we found that LPS-stimulation of B cells either in the presence or absence of factors from A32-26 cells developed a similar number of IgM PFC, indicating that these factors did not inhibit IgM secretion or plaque formation. We conclude that on stimulation by Con A the T hybridoma A32-26 cells produce BRF but not BMF. Judging by replication in the presence of A32-26 BRF and LPS, A32-26 BRF stimulates polyclonal activation of B-cell blasts. This polyclonal restimulation is also independent of the H-2 or immunoglobulin haplotype of the B-blasts, since B-blasts of C57BL/6J *nu/nu* mice (Ig^b, H-2^b), BALB/c *nu/nu* mice (Ig^a, H-2^d) and A/J mice (Ig^e, H-2$^{k/d}$) could all be restimulated. This is in line with earlier observations, that neither surface immunoglobulin nor surface Ia have to be occupied by ligands to allow BRF to stimulate B-blast through the next round of division.

The activity of helper T cells in T-dependent B-cell stimulation can be replaced by soluble factors (TRF) obtained in the interaction of helper T cells, antigen and macrophages[8–15]. Thus, B cells of athymic *nu/nu* mice will respond to antigens such as sheep erythrocytes (SRBC) by clonal replication and maturation to secrete SRBC-specific antibody in the presence of such TRF. Factors from T hybridoma A32-26 have no activity detectable as TRF, while other media expected to contain TRF, do, indeed show activity (Table 1). We think that this is because they do not contain BMF which could induce SRBC-specific replicating B cells to cells secreting SRBC-specific antibody, detectable as PFC.

The apparent molecular weight of BRF produced by the A32-26 T hybridoma cells was estimated by gel filtration over a G100-Sephadex column. BRF is detectable in two major molecular forms, with apparent molecular weights of 80,000–90,000 and 15,000–20,000, and two minor forms with molecular weights of ~45,000 and >100,000 (Fig. 2). Both human and murine BRF have also recently been identified in other laboratories[5,26]. Structural studies on the chemical nature of the BRF from A32-26 T hybridoma cells and from other BRF-producing hybridomas should clarify whether the different

Table 1 Proliferation of T- and B-cell blasts in media conditioned by factors from different sources

Medium conditioned with	CTL-L killer T cells‡	Helper T cells#	LPS-activated B-blasts§	SRBC-specific PFC per 2×10^6 *nu/nu* C57BL/6J cultured spleen cells**	Colonies per 10^5 bone marrow cells††
	($10^{-3}\times{}^3$H c.p.m thymidine uptake per 2×10^4 plated cells)				
	TCGF	TCGF	BRMF	TRF	CSF
Con A-activated T-hybridoma A32-26 cells*	0, 15	0, 18	*15, 0*	<25	0
Helper T cells‖	*7, 5*	*11, 5*	*6, 0*	*9,500*	*51*
LPS¶	0, 15	0, 2	*10, 0*	*2,000*	0
Con A-activated spleen cells†	*15, 0*	*14, 0*	*14, 0*	*15,000*	ND
None	0, 15	0, 15	0, 8	<25	0
Con A-activated BW 5147 cells*	0, 1	0, 1	0, 3	<25	0
Con A	0, 1	0, 1	0, 2	<25	0
WEHI-3 cells	0, 1	0, 1	ND	<25	*90*

* T hybridoma and BW5147 cells were stimulated at 5×10^5 cells per ml for 24 h with 5 μg ml^{-1} Con A in serum-free medium[33] in a CO_2-incubator at 9% CO_2 in air The conditioned media were used at 25% (except the Con A-activated spleen cell superniatants, see below), in the presence of 5mM α-methylmannoside (Sigma). All *in vitro* experiments described here were done in serum-free medium[33].

† 5×10^6 rat spleen cells per ml incubated with 5 μg ml^{-1} Con A for 24 h, supernatant medium concentrated by ammonium sulphate precipitation and partially purified by G100-Sephadex chromatography. Used at 10%.

‡ The CTL-L killer T-cell line was a gift of Dr K. Smith 5×10^4 CTL-L cells per ml were cultured in the conditioned media for 48 h, then 1 μCi ^{3}H-thymidine (2Ci mmol^{-1}, Amersham) was added for 2 h and the incorporation into insoluble material measured as described[33].

§ C57BL/6J *nu/nu* spleen cells activated for 48 h with 50 μg ml^{-1} LPS, partially purified from non-activated and from dead cells by velocity sedimentation[34] as described previously[21] Thymidine uptake as for CTL-L cells

‖ Influenza virus-specific helper T-cell clone vir2-15 (ref. 23), incubated at 2×10^5 cells per ml with 10 haemagglutinating units of PR8 virus and 2×10^6 2,200 rad X-irradiated BALB/c spleen cells.

¶ 50 μg/ml.

Helper T cells were the cloned, influenza virus-specific cell line vir2-15, kept on virus and syngeneic irradiated spleen cells as described[25], partially purified from dead cells and virus before use by repeated sedimentation over a layer of fetal calf serum. Thymidine uptake was done as with CTL-L cells (see ‡).

** Culture containing 2.5×10^6 SRBC (sheep erythrocytes) and 2×10^6 *nu/nu* C57BL/6J spleen cells, assayed at day 5 of culture as described previously[1,19].

†† Colony stimulating factor (CSF) activity was detected in methylcellulose culture by growth of colonies (macrophage, monocyte, granulocyte and erythrocyte colonies) from bone marrow precursor cells[35] as described previously[36].

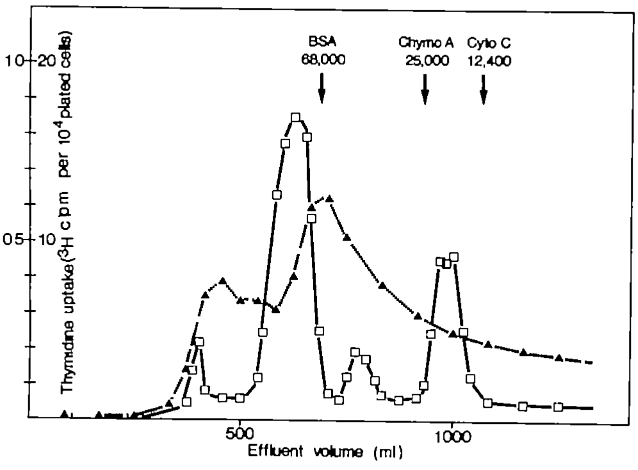

Fig. 2 Gel filtration on G100-Sephadex (column size 5 × 90 cm) of BRF produced by Con A-activated A32-26 T hybridoma cells (for details see Table 1). BRF from 1 l of conditioned medium concentrated by precipitation with ammonium sulphate (80% saturation) and dialysed against the column buffer (0.9% NaCl, 5 mM HEPES pH 7.3) was applied to the column at 4 °C. The eluate collected in 10-ml fractions was monitored for absorbance at 280 µm (···▲···) and for BRF activity at 3% in culture (—□—) as described in Table 1 legend. The same column, in a parallel run, was calibrated with BSA (molecular weight 68,000), chymotrypsinogen A (25,000) and cytochrome *c* (12,400).

molecular species are structurally different, or whether they share common structural elements involved in BRF action, derived through a precursor–product relationship[27–29], in a free factor/receptor-bound factor relationship[30,31] known to exist for growth factors of other eukaryotic cells or, more trivially, by factors bound to molecules of the tissue culture medium such as bovine serum albumin.

The finding that BRF and BMF are separate activities has important bearing on our understanding of the mechanisms and the physiology of B cell regulation. Resting B cells might express BMF but not BRF receptors, while excited B cells might express both. The establishment of continuous B-cell lines and clones, analogous to functional T-cell lines and clones[32] has so far been difficult. We hope that the stimulation by BRF in the absence of BMF of excited B cells not yet induced to mature will help in this experimental difficulty. Replication of B lymphocytes in the presence of BRF may continue for longer periods of time as long as the terminating action of BMF is absent.

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LFA-1 but not Lyt-2 is associated with killing activity of cytotoxic T lymphocyte hybridomas

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The mechanism by which cytotoxic T lymphocytes (CTL) recognize and lyse target cells is largely unknown. Recently, two lymphocyte cell-surface glycoproteins, Lyt-2[1–4] and LFA-1 (lymphocyte function-associated antigen)[5–8], have been implicated in the killing activity of murine CTL. This suggestion was based mainly on the unique ability of antibodies directed against Lyt-2[1–4] and LFA-1[5–8] to inhibit cytotoxicity of CTL by affecting the killer cell. Lyt-2, a glycoprotein composed of subunits of molecular weights of 30,000–35,000 (30–35K), has been proposed as a common marker for CTL[9], essential for specific recognition of target cells[10]. This view, however, is incompatible with other results[11–14]. The newly detected LFA-1 molecule, containing subunits of 95K and 180K[5–8,15], is associated with the ability of CTL to bind target cells[16,17]. Most analyses of CTL structure–function relationships are ambiguous since they have been obtained with heterogeneous CTL populations. We have recently developed CTL-hybridomas which lyse specifically allogeneic tumour cells *in vitro*[18] and grow independently of exogenous stimuli[19]. In this study we have tested the relevance of Lyt-2 and LFA-1 antigens to cytotoxicity by analysing the CTL-hybridomas with a panel of anti-LFA-1 and anti-Lyt-2 monoclonal antibodies. We show that two monoclonal CTL-hybridomas have the phenotype LFA-1$^+$ and Lyt-2$^-$ and that their killing activity is inhibited by anti-LFA-1 but not by anti-Lyt-2. The results suggest that LFA-1 is involved in CTL-cytotoxicity, while Lyt-2 molecules are not required.

CTL somatic hybrids, in which elimination of functionally irrelevant molecules could occur due to chromosomal segregation or to repression controlled by the tumour cell parent, provide a new homogeneous and antigen-specific source for

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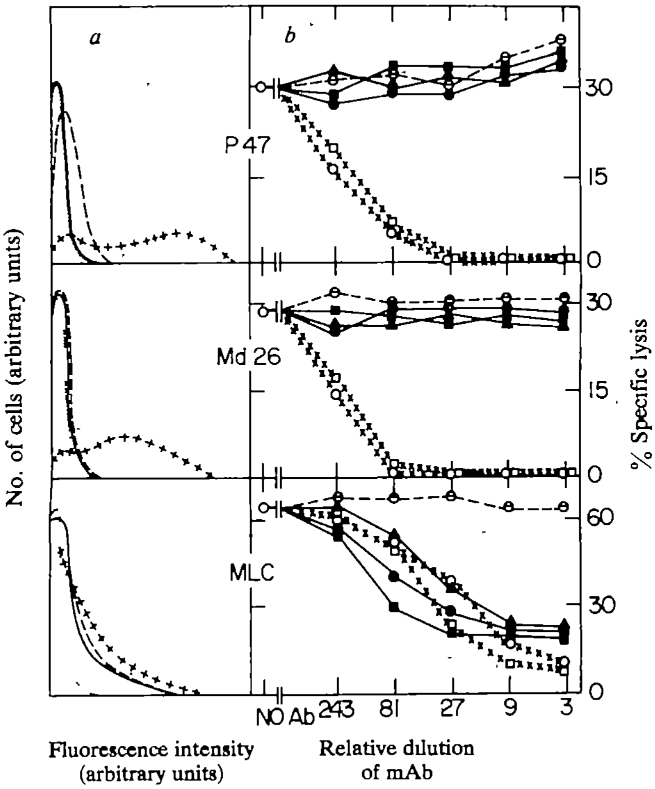

Fig. 1 Expression of Lyt and LFA-1 antigens on CTL-hybridomas and the effect of anti-Lyt and anti-LFA-1 mAb on cytotoxicity. *a*, Cytofluorometric analysis of Lyt-1, Lyt-2 and LFA-1 on CTL and CTL-hybridomas was done by reacting 2×10^6 MLC effector cells (primary BALB/c anti-C57BL/6 spleen cells stimulated for 5 days at 1:1 ratio) or CTL-hybridoma clones P.47 or Md.26 with the following monoclonal antibodies. anti-Lyt-1 (- - - -) and anti-Lyt-2 (——) produced by hybridomas 53.7.3 and 53.6.7, respectively[22] (these were obtained from the Cell Distribution Center of the Salk Institute, San Diego); anti-LFA-1 (+++) produced by hybridoma H35-89.9[7] and concentrated from ascites by 50% $(NH_4)_2SO_4$. Washed cells were stained with fluoresceinated rabbit anti-rat immunoglobin. Samples were analysed using a fluorescence-activated cell sorter (FACS II). For each cell preparation, a control staining with the fluoresceinated reagent alone (· · · ·) is shown. Data are expressed in arbitrary units as obtained from the cell sorter. *b*, The effect of anti-Lyt and anti-LFA-1 monoclonal antibodies on cytotoxicity of MLC and CTL hybridomas was studied by incubating 10^4 effector cells in microtitre plates with different dilutions of the following monoclonal antibodies: anti-Lyt-1, 53.7.3[22] five times concentrated (- - - - ○); anti-Lyt-2 were 53.6.7[22] five times concentrated (▲); H35-17.2[17] 1:3 diluted (●); and M12/7.2[5] 1:10 diluted (■); anti-LFA-1 were H35-89.9[7] at 1:1,000 dilution (+++○); and M17/5.2[5] at 1:30 dilution (+++□). The concentrations of antibodies listed above indicate the original stocks which were serially diluted in the assay. After 20 min incubation at 37 °C in 100 µl reaction medium (RPMI-containing 10% fetal calf serum and 10 mM HEPES *p*H 7.4), 10^4 ^{51}Cr-labelled, neuraminidase-treated[18] EL4 target cells were added in 50 µl reaction medium. Plates were spun for 5 min at 500 r.p.m. and incubated for 5 h at 37 °C. Lysis was stopped by cooling, plates were spun for 10 min at 2,000 r.p.m. and 75 µl supernatants were taken to determine the ^{51}Cr release. Spontaneous release was 12%.

studies of specific effector structures participating in cytotoxicity. We analysed the effect of various monoclonal antibodies on two unrelated CTL-hybrid subclones, Md. 26 and P. 47, that had been derived from BALB/c anti-EL4 (H-2^d anti-H-2^b) CTL following secondary stimulation either in mixed lymphocyte culture (MLC) or *in vivo* (peritoneal exudate lymphocytes, PEL), respectively[18]. These monoclonal CTL-hybridomas retained their killing specificity towards H-2^b tumour target cells during prolonged continuous growth in culture[20] and exhibited this capacity while the studies described here were carried out. The monoclonal antibodies examined had been raised against CTL and selected according to their ability to inhibit killing by binding to the effector cells[5,7,17,21].

The presence of the differentiation antigens Lyt-1, Lyt-2 and LFA-1 on the CTL hybridomas and heterogeneous MLC population was examined by immunofluorescence staining. The analysis (Fig. 1*a*) demonstrates the presence of all three antigens on MLC, low levels of Lyt-1 on P but not on M hybridomas and high density of LFA-1 on both hybridomas. None of the CTL-hybridomas were stained with anti-Lyt-2. Additional monoclonal anti-Lyt-2 (H35-17.2[7,17], M12/4, M12/5 and M12/7.2[21]), failed to stain the hybridomas, while several anti-LFA-1 monoclonal antibodies (H35-89.9[7] and M17.5.2[5]) stained over 85% of the hybrid cells (not shown). The relevance of Lyt-2 and LFA-1 to the killing activity was further studied by testing the effect of the monoclonal antibodies on cytotoxicity (Fig. 1*b*). The hybridomas or BALB/c anti-C57BL/6 MLC were incubated with ^{51}Cr-labelled EL4 target cells in the presence of diluted antibody and specific killing was determined. As previously reported, cytotoxicity of MLC was inhibited by anti-Lyt-2[1–4] and by anti-LFA-1[5–8] and was unaffected by anti-Lyt-1. The killing activity of both CTL-hybridomas was likewise unaffected by anti-Lyt-1 and completely abolished by two anti-LFA-1 antibodies. However, unlike parental MLC, the cytotoxicity of the hybridomas was unaffected by three different anti-Lyt-2 monoclonal antibodies. The absence of detectable Lyt-2 on the surface of the CTL-hybridoma was further confirmed by preincubation of both M and P hybrid cells with two different anti-Lyt-2 antibodies in the presence of complement (Fig. 2). Such pretreatment completely eliminated the cytotoxicity of parental MLC but did not affect the cytolytic potential of hybridomas. The inability to detect Lyt-2 on the

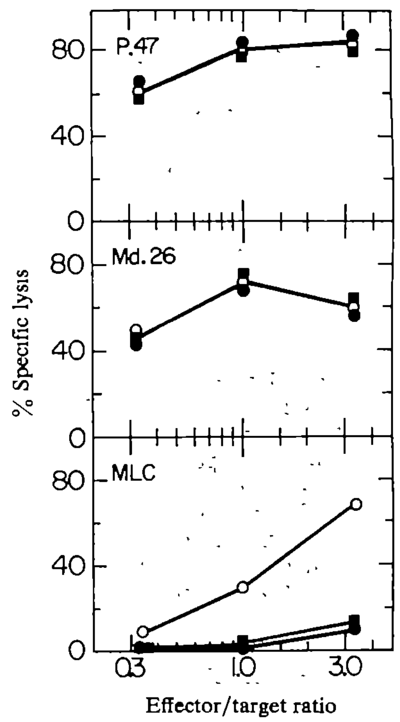

Fig. 2 The effect of pretreatments with anti-Lyt-2 and complement on the cytotoxicity of CTL-hybridomas and MLC. CTL-hybridomas and primary MLC (see Fig. 1 legend) were preincubated for 45 min at 37 °C with anti-Lyt-2, H35-17.2[17] (●) or M12.7.2[5] (■) and 1:10 dilution of fresh guinea pig serum as complement or with complement alone (○). After washing, treated cells were diluted and cytotoxicity was tested on 10^4 neuraminidase-treated ^{51}Cr-labelled EL4 target cells. E/T ratio was calculated according to number of effector cells before treatment. Incubation was for 5.5 h and spontaneous release was 18%.

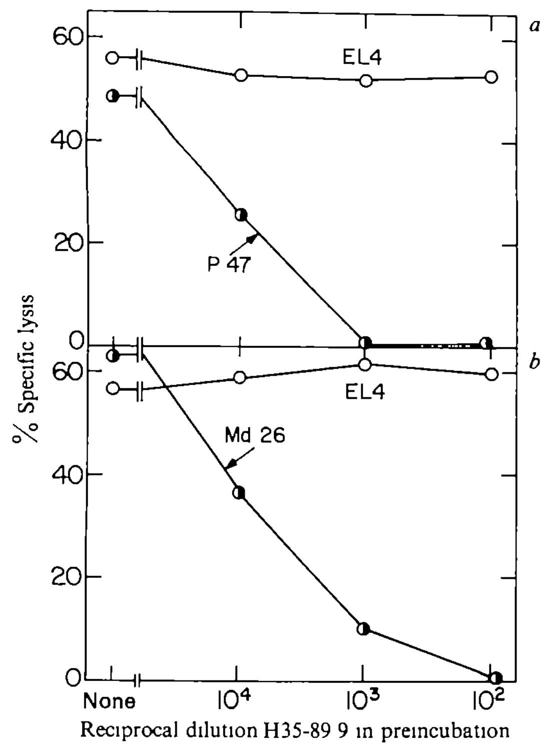

Fig. 3 Preincubation of CTL-hybridomas but not of EL4 target cells with monoclonal anti-LFA-1 inhibits cytotoxicity. Isolated CTL-hybridomas or neuraminidase-treated ^{51}Cr-labelled EL4 cells were incubated with anti-LFA-1 H35-89.9[7] at the indicated dilutions for 60 min at 37 °C. Cells were washed three times from excess of monoclonal antibody and cytolytic reactions were completed with an equivalent number of the missing untreated effector or target cells. Killing assays contained 10^4 CTL-hybridomas (either P.47 (*a*) or Md.26 (*b*)).

CTL hybridomas did not result from a wrong specificity or a low titre of the antibody used; since antibody 53.6, which recognizes a nonpolymorphic portion of Lyt-2 molecule[22] and two other monoclonal anti-Lyt-2 molecules, did bind to parental MLC killers and inhibited very effectively their cytotoxicity, while the same monoclonal antibodies could neither bind to the hybrid cells (Figs 1*a* and 2) nor affect their killing activity (Fig. 1*b*). Furthermore, more than a single Lyt-2 antigenic determinant was absent, since the hybridomas were unreactive with antibodies M12/4, M12/5 and M12/7, which define three topographically distinct Lyt-2 determinants (not shown).

In contrast to anti-Lyt-2, four different monoclonal anti-LFA-1 molecules were all equally effective in inhibiting specific killing of EL4 and ALB.B2 target cells by the hybridomas (Fig. 1*b* and not shown). Since LFA-1 molecules are expressed not only on the CTL-hybridomas but also on EL4 (ref. 15 and our unpublished results), which had been used as target cells in these experiments, it was necessary to examine whether anti-LFA-1 exerts its inhibitory effect on killing through its binding to the target cells or to the effector cells, as previously suggested[5-8]. This was tested by preincubating anti-LFA-1 separately with either isolated CTL-hybridomas or EL4 cells and combining treated and untreated components for the killing assay after excess of the antibody was removed. Cytotoxicity of preincubated CTL hybridomas was completely abolished by bound anti-LFA-1 molecules (Fig. 3), while pretreated EL4 target cells were unaffected, showing that indeed, anti-LFA-1 inhibited killing by affecting primarily the CTL-hybridomas rather than EL4 target cells.

The hypothesis that Lyt-2 is associated with the CTL receptor was based on a genetic linkage between Lyt-2-3 locus and the V_k locus[23], on the observation that anti-Lyt-2 inhibits killing at the recognition-adhesion stage[24], and on a mutagenized CTL Lyt-2$^+$ clone which lost in parallel Lyt-2 and the ability to kill specifically TC[10]. Recently, however, MacDonald *et al.*[14] reported that some CTL which express Lyt-2 are not inhibited by anti-Lyt-2. In addition, it becomes apparent that CTL populations[12] and long-terms lines[11,13] directed against Ia antigens (class II) major histocompatibility complex (MHC) are Lyt-1$^+$2$^\mp$ and helper cells specific for class I MHC antigens are Lyt-1$^+$2$^+$ and their helper function is inhibited by anti-Lyt-2[26]. These results prompted Swain[26] to suggest that Lyt phenotype correlates primarily with the class of MHC antigen recognized rather than with function (see also ref. 27 for a recent discussion). Our observation that functional CTL-hybridomas are Lyt-2 negative support the notion that Lyt-2 molecules are not required for specific cytotoxicity. The H-2 specificity of these CTL-hybridomas was recently mapped to the *D* region of *H-2*^{b} by using target cells from recombinant mice[28] and by inhibition of the hybridoma-mediated target cell killing with anti-H-2D^b[19]. In this respect, the CTL-hybridomas seem to be exceptional, since all CTL directed against class I MHC were reported so far as Lyt-2$^+$. However, hybridomas are often subjected to selective loss of some parental chromosomes or to repression of specific genes, hence it is possible that the CTL hybridomas analysed here originated from Lyt-2$^+$ parental CTL whose Lyt-2 expression was lost. Regardless of the history of these cells, the mere fact that they specifically lyse target cells without expressing Lyt-2 antigens strongly suggests that Lyt-2 molecules are not essential for either target cell recognition or binding. This is in contrast to previous suggestions that Lyt-2 is an antigen receptor. However, it is possible that Lyt-2 is a non-antigen-specific receptor that, together with the antigen receptor, contributes to the avidity of the CTL for the target cells[17,21]. In cases where the antigen receptor has low avidity, loss of the Lyt-2 interaction would result in loss of CTL activity, explaining the antibody inhibition[1-4] and Lyt-2 mutant results[10]. Thus, while in some cases Lyt-2 may contribute to CTL activity, we conclude that it is not essential and is distinct from the antigen receptor.

LFA-1 molecules, unlike Lyt-2, may be an essential structure of functional CTL. This conclusion is based on the observation that *all* CTL populations and clones examined so far express LFA-1 and their cytotoxicity is inhibited by anti-LFA-1[5-8]. The formation of effector target cell conjugates is inhibited by anti-LFA-1[16,17], indicating that the binding stage is affected. The ability of anti-LFA-1 to inhibit specific lysis irrespective of the TC specificity[7,16], as well as concanavalin A mediated nonspecific killing[7,17], emphasizes the general role of LFA-1 in a fruitful CTL target cell interaction. Previous findings that anti-LFA-1 interfere with other T-cell functions like proliferative MLC responses, macrophage-dependent antigen-specific proliferation, and T cell-dependent antibody responses to red cells[7,16,21], suggest that LFA-1 is crucial for the interaction of T cells with other cells. The fact that a panel of anti-LFA-1 monoclonal antibodies inhibited very efficiently the specific cytotoxicity of CTL-hybridomas (Figs 1*b*, 3) and immunoprecipitated from the hybrid cells the 95K and 180K LFA-1 subunits (not shown) indicates that the same LFA-1 molecules that participate in parental CTL cytotoxicity also participate in the specific target cell killing induced by the hybridomas. The monoclonal CTL hybridomas which grow rapidly in culture and retain specific killing activity for months without added stimuli, provide an unlimited and homogeneous source for studying the molecular basis for the role of LFA-1 in T cell-mediated cytotoxicity.

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Restricted expression of viral glycoprotein in cells of persistently infected mice

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For a virus to cause persistent infection in a host, a unique host–virus relationship is required. The virus *per se*, or variant(s) arising *in vivo*, must be noncytopathic for the host cell that it infects. Furthermore, the infected cell must escape recognition by the host's immune system, which eliminates virus and virus-infected cells. Several models of persistent infection *in vitro* demonstrate selective loss of viral gene products normally represented at the cell surface as opposed to viral products found inside the cell[1–5]. Decreased glycoprotein (gp) expression on the surface of an infected cell correlates directly with the ability of that cell to resist killing by both cytotoxic lymphocytes and cytotoxic antibody and complement[1,3,6]. The question remains of whether a corresponding selective loss of viral glycoprotein expression occurs in persistently infected cells *in vivo*. We have used monoclonal antibodies as probes to define the viral glycoprotein and nucleoprotein (NP) molecules expressed in individual cells from several tissues of mice persistently infected with lymphocytic choriomeningitis virus (LCMV). We report here a selective decrease in the expression of viral glycoprotein in single cells, during persistent infection *in vivo*.

LCMV is noncytopathic and easily induces persistent infection in its natural host, the mouse (for review see refs 7, 8). The virus contains a negative-stranded two-segmented RNA genome and three structural polypeptides. Of these, two are glycosylated (gp1 and gp2), and one is the nonglycosylated NP; the molecular weights are 4.3×10^4, 3.6×10^4 and 6.3×10^4 for gp1, gp2 and NP, respectively[7]. Collectively, the glycoproteins make up ~30% of the virion mass. The gp1 and gp2 form in infected cells in equimolar amounts by proteolytic cleavage from a common precursor, gpC, and are distinct according to tryptic peptide mapping[9]; gp1 is the predominant viral polypeptide accessible to radiolabelling or antibody binding on the surfaces of infected cells or virions[9]. A large battery of monoclonal antibodies against these three structural LCMV polypeptides was obtained by using a modification of the Kohler–Milstein method[10]. Specificities of the various monoclonal antibodies were then determined after immune precipitation of ^{35}S-labelled LCMV polypeptides, as described elsewhere[10]. The antibodies were used as molecular probes to analyse the expression of gp1, gp2 and NP by indirect immunofluorescence assay[6,10].

We first determined the expression of viral gene products during acute LCMV infection of cultured cells and subsequently of intact, adult animals. Viral gp1, gp2 and NP were evident in the cytoplasm of both L929 and BALB/clone (Cl) 7 cells 2 days after initiating infection (see Fig. 1). Similar results were obtained when either hybridoma cells or N115 neuroblastoma cells were similarly infected and analysed for each viral polypeptide, or when individual cells were stained with two fluorochrome dyes[11] (one dye was used to detect the NP, the other to detect viral glycoproteins). For these and other studies, the antiviral gp1 and gp2 monoclonal antibodies were pooled and used as a reference glycoprotein reagent. The plasma membrane showed expression of molecules of viral glycoprotein only on the surfaces of acutely infected cells. In subsequent tests, ependyma, choroid and meningeal cells from several individual adult mice of the C3H/St, BALB/WEHI and SWR/J strains acutely infected with LCMV (60 plaque-forming units (PFU), i.c.) expressed both types of viral glycoprotein and NP when sampled 5 days after infection (Fig. 2).

In contrast to our findings in acutely infected mice and cells, mice persistently infected *in vivo*, as well as cultured mouse cells persistently infected *in vitro* with LCMV, selectively lost viral gp expression with time but retained viral NP. For example,

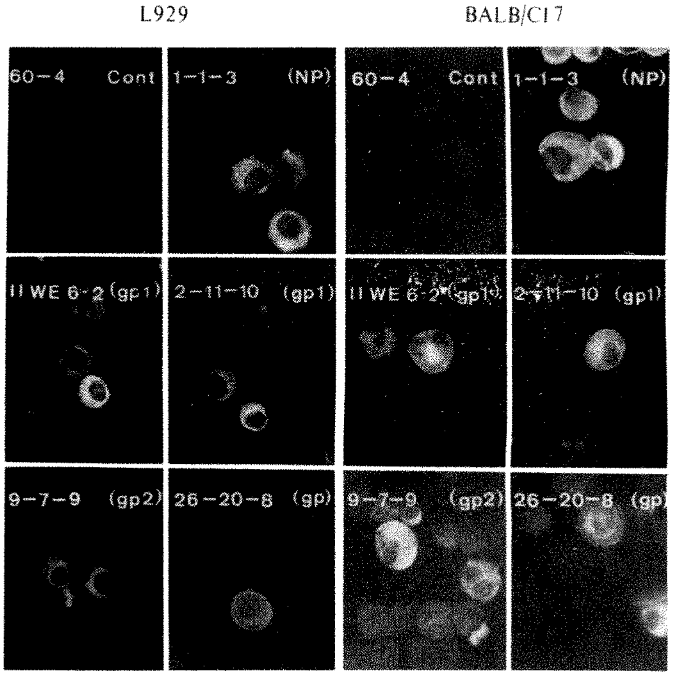

Fig. 1 Expression of viral glycoprotein and NP by cultured cells during acute infection with LCMV, Armstrong 1371 strain. L929 or BALB/Cl 7 cells were infected with LCMV at a multiplicity of infection (MOI) of 3 and studied 2 days later for the expression of viral gp1, gp2 or NP. Cells were fixed with ether/alcohol (1 : 1) for 20 min, 95% ethanol for 10 min, then washed in buffer and stained with the monoclonal antibodies shown. 60–4 is a monoclonal antibody to the glycoprotein (G) protein of vesicular stomatitis virus and served as a negative control. After 30 min incubation, the cells were washed twice in buffer and stained with monospecific goat anti-mouse immunoglobulin antibody conjugated to rhodamine. After 20 min, the cells were triple-washed in buffer, covered with glycerol–Tris buffer and a coverslip and studied by fluorescence microscopy.

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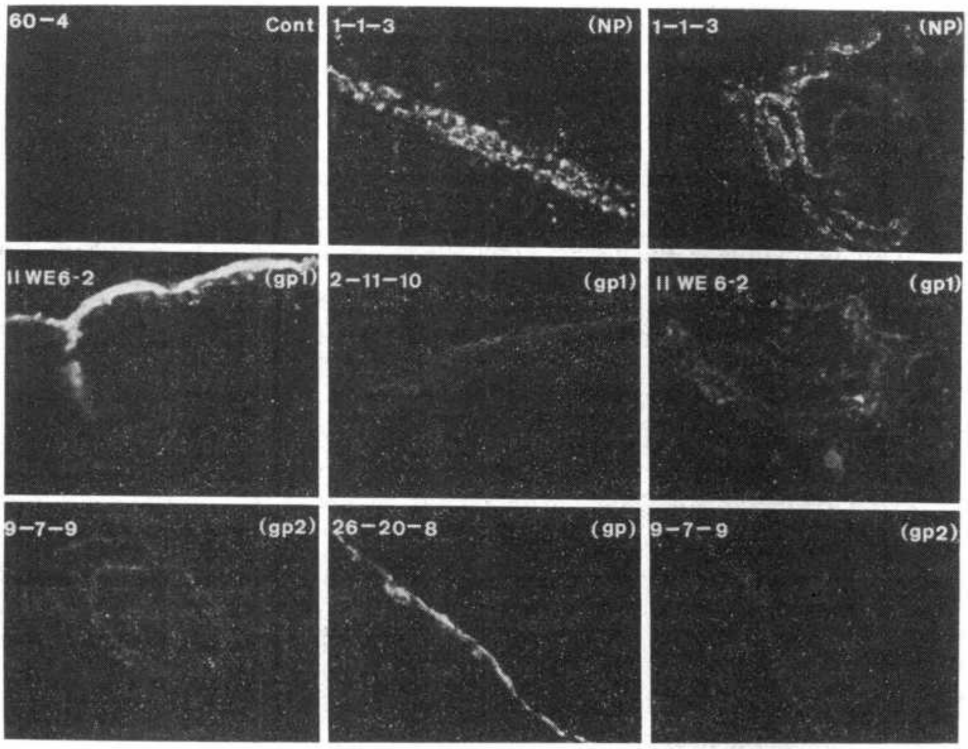

Fig. 2 Expression of viral glycoprotein and NP during acute infection of adult C3H/St mice with 60 PFU of LCMV, Armstrong 1371 strain. Expression of both viral glycoprotein and NP is evident in cells of the ependyma (centre—upper row), choroid plexus (right—upper row; right—middle row; left—lower row) and meninges (left and centre—middle row; centre and right—lower row). Brains collected 5 days after infection were snap-frozen with liquid nitrogen, cut into 4-μm sections in a cryostat and mounted on glass slides. Slides containing the brain sections were fixed and stained as described in Fig. 1 legend. Similar results were seen when anti-gp1 and -gp2 monoclonal antibodies were pooled and then used as a staining reagent. Similar results were observed with SWR/J and BALB/W mice.

at 60 days after the initiating infection, and for as long as 360 days after the experiment ended, persistently infected cells expressed no detectable viral gp on their surfaces and only rarely was it found in the cytoplasm. This observation has been confirmed in studies of single cells from animals infected *in vivo* (Fig. 3 and Table 1). For the *in vivo* study, persistent infection was established by inoculating newborn SWR/J, BALB/WEHI or C3H/St mice i.c. with 60 PFU of LCMV, Armstrong 1371 strain[12]. Such neonatally infected mice carry virus in their tissues throughout life despite mounting an antiviral immune response[7,12]. We evaluated cells from the brain, liver, kidney and pituitary tissues of such mice at 2, 5, 15, 30, 60, 84 and 180 days after initiating infection, for expression of viral glycoprotein or NP, using monoclonal antibodies (Table 1). At the second and fifth days after infection, neurones of the central nervous system (CNS), parenchymal cells of the liver, tubular epithelial cells of the kidney and cells in the anterior lobe of the pituitary expressed viral glycoprotein and NP in a manner similar to acutely infected cells from adult mice (Fig. 2) and acutely infected cells in culture (Fig. 1). By 15 days after infection, most but not all infected cells demonstrated both glycoprotein and NP polypeptides. However, at 30 days and later, individual infected cells located in the CNS, liver, kidney and anterior lobe of the pituitary had lost viral glycoprotein but retained the expression of viral NP (Fig. 3, Table 1).

Table 1 Dissociated expression of LCMV glycoprotein and NP during persistent virus infection

		Expression of viral gene products in neurones							
		Acute infection		Persistent infection					
		Day 3–5		Day 5		Day 15		Day > 80	
Strain		gp	NP	gp	NP	gp	NP	gp	NP
C3H/St	Adult	8/8[*]	8/8	—[†]	—	—	—	—	—
BALB/W	Adult	5/5	5/5	—	—	—	—	—	—
SWR/J	Adult	8/8	8/8	—	—	—	—	—	—
C3H/St	Newborn	—	—	5/5[*]	5/5	9/15	15/15	1/8	8/8
BALB/W	Newborn	—	—	5/5	5/5	ND	ND	1/20	20/20
SWR/J	Newborn	—	—	5/5	5/5	ND	ND	3/25	25/25

Individual neurones were studied for the expression of viral glycoprotein (gp) or NP by using appropriate monoclonal antibody and antibody to mouse immunoglobulin conjugated to rhodamine or fluorescein isothiocyanate. Sections (4 μm) through the anterior one-third of the temporal lobe were fixed in ether/alchohol (1 : 1) and 95% alcohol before staining as described elsewhere[12]. At least 100 individual neurones per section were studied. Acute infection was induced in 4–5-week-old mice by i.c. inoculation of 60 PFU of LCMV, Armstrong strain 1371; persistent infection followed inoculation of newborn mice with a similar route of inoculation and dose of virus. Infected mice were killed after infection, on the days indicated. ND, not determined.

[*] Number of mice studied that contained one or more cells expressing a specific viral polypeptide/total number of mice.

[†] The virus dose used in adult mice for acute infection leads to death by day 6–8.

These studies suggest that the synthesis of viral glycoprotein decreases in cells during persistent infection *in vivo*, resulting in a decrease in its expression on the surfaces of the cells. Decreased viral glycoprotein expression possibly allows persistently infected cells to resist assault by virus-specific immune reactants. By this means, a noncytopathic virus may persist throughout the lifespan of an infected cell and the cell could remain alive, unaffected by the host immune surveillance mechanism.

Although the cellular content of viral glycoprotein diminished, that of viral NP did not. Despite the accumulation of viral NP in specialized cells of the host, no morphological alteration was evident by either light or electron microscopy[13,14]. Further, electron microscopic study of infected neuronal cells from persistently infected mice reacted with monoclonal antibodies confirmed that NP accumulated inside the cell, usually aggregated in the area of polyribosomes[14], while viral gp inside the cell was reduced and the virus became restricted from budding at the cell surface.

These findings in individual neuronal, hepatic, epithelial and endocrine cells from mice persistently infected with LCMV *in vivo* extend the results seen *in vitro*[1–5,15,16]. Such persistently infected cells develop a quantitative 10- to 50-fold decrease in viral glycoprotein synthesis and glycoprotein expression compared to NP synthesis and expression in acutely infected cells[1,15]. Our *in vitro* studies suggest that the decrease in glycoprotein expression parallels the generation of defective interfering virus (DIV)[1,15]. Whether DIV is generated *in vivo* during natural or experimentally induced LCMV infection is unknown, but is now being studied.

Persistent LCMV infection of cultured differentiated neuroblastoma cells correlates directly with a significant dysfunction in the synthesis or degradation of acetylcholine due to the reduction in acetylcholine transferase or esterase, respectively[16]. Similar alterations in differentiated functions have

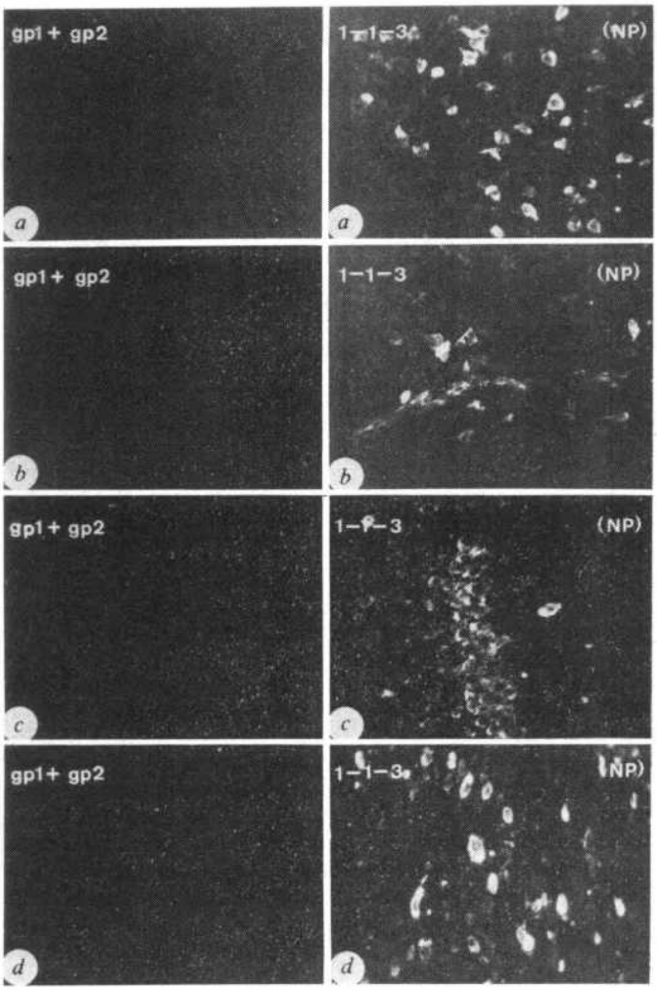

Fig. 3 Lack of viral glycoprotein but presence of NP in individual neuronal cells from C3H/St mice persistently infected with LCMV. Newborn mice were inoculated with 60 PFU of LCMV, Armstrong 1371 strain and killed 84 days later. Neuronal cells of four mice (*a–d*) expressed NP, but in the adjunct 4-μm section no glycoprotein is apparent for the same cell. The collection, sectioning and staining of the tissues was the same as described in Fig. 2 legend, except that antibodies to gp1 and gp2 from hybridomas were pooled and then used as the staining reagent. Similar results were observed for SWR/J and BALB/W mice.

recently been observed after viral infection *in vitro* when hybridoma cells no longer produced monoclonal antibodies, and *in vivo* when differentiated growth hormone-producing cells made significantly less growth hormone[3,17]. Hence, by avoiding immune responses directed against the cells they infect, noncytopathic viruses may significantly alter physiological homeostasis by causing a dysfunction in the cells of the endocrine, immune or other differentiated systems; the end result is a new mechanism for the production of virus-induced diseases. Whether these biochemical dysfunctions at the cellular level are directly related to the accumulation of viral products in a manner akin to a storage disease is a matter for further study. The results reported here for persistent LCMV infection resemble the overall pattern observed during persistent measles virus infection (subacute sclerosing panencephalitis). Neuronal cells in biopsies from these patients showed a decrease in or lack of measles virus glycoprotein but they accumulated measles virus nucleocapsids internally[18]. Thus, other persistent infections may produce similar findings *in vivo*.

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DNA strand breaks and ADP-ribosyl transferase activation during cell differentiation

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The nuclear enzyme ADP-ribosyl transferase (ADPRT) catalyses the formation of poly(ADP-ribose)-modified chromatin proteins from NAD$^+$ (refs 1–5) and is entirely dependent on DNA[6] containing nicks[7–11]. Nuclear ADPRT activity is required for efficient DNA excision repair[12,13], probably because it regulates DNA ligase activity[14]. Indirect evidence has suggested that ADPRT activity may also be involved in control of gene expression and cell differentiation[15–21]. We report here an obligatory involvement of ADPRT activity in the differentiation of muscle cells. Inhibitors of ADPRT activity reversibly inhibit both fusion of myoblasts to form multi-nucleate muscle fibres and the differentiation-specific increase in creatine phosphokinase (CPK) activity. These two markers of differentiation can also be reversibly inhibited by depriving the cells of nicotinamide and thus lowering their cellular NAD content. Specific gene expression sometimes requires gene rearrangements or DNA transposition; this implies that DNA strand-breaking and rejoining might be involved in gene expression. We also describe the appearance during cytodifferentiation of single-strand DNA breaks which are not due to a general deficiency in DNA repair.

Primary chick myoblasts in culture undergo an initial phase of proliferation before terminal differentiation which is marked by the fusion of myoblast cell membranes to form multinucleate syncytia of muscle fibres, the cessation of DNA biosynthesis and cell division, and a substantial increase in CPK activity, largely due to *de novo* synthesis of a muscle-specific isozyme of this protein (Fig. 1).

ADPRT activity can be estimated by the rate of incorporation of radioactivity from ^{3}H-adenine-NAD$^+$ into acid-insoluble material in permeabilized cells. Two forms of ADPRT activity may be measured[10]; the intrinsic, physiological, endogeneous activity in the absence of any experimentally induced DNA strand breaks, and the total, potential enzyme activity displayed by deoxyribonuclease to generate the optimal number of breaks.

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During the initial, proliferative phase of culture there is a low, constant physiological ADPRT activity (Fig. 1). Just before the onset of cell fusion, however, the physiological activity begins to rise, most of the increase taking place before 45 h. By 72 h, when ~70% of the cells have fused, the specific activity of ADPRT has increased threefold (Fig. 1). In contrast, the total potential ADPRT activity is higher than the physiological activity at all times and does not alter significantly (Fig. 1), implying that the increase in the physiological activity is due to enzyme activation, and not to an increase in the number of enzyme molecules. ADPRT activation precedes both cell fusion and the rise in CPK activity (Figs 1, 2*b*, *d*).

We have analysed the function of ADPRT by the use of enzyme inhibitors[13]. In the continuous presence of either 3-aminobenzamide or 3-methoxybenzamide[22], myoblast fusion is inhibited in a concentration-dependent manner. 3-Aminobenzamide (8 mM) or 3-methoxybenzamide (5 mM) inhibit myoblast fusion essentially completely, myoblast proliferation is not inhibited (results not shown, but see Fig. 2). In contrast, the non-inhibitory analogues 3-aminobenzoate and 3-methoxybenzoate cause no significant inhibition of fusion at corresponding concentrations. Inhibitors of ADPRT block reversibly both myoblast fusion (Fig. 2*a*) and the usual increase in CPK activity (Fig. 2*b*). The replacement of 3-aminobenzamide-containing medium with medium from control cultures of the same age, at 44 or 54 h of culture, reversed the inhibition and cell fusion began 10 or 14 h later respectively (Fig. 2*a*). Removal of the inhibitor at 54 h of culture results in a rise in CPK activity 12 h later (Fig. 2*b*).

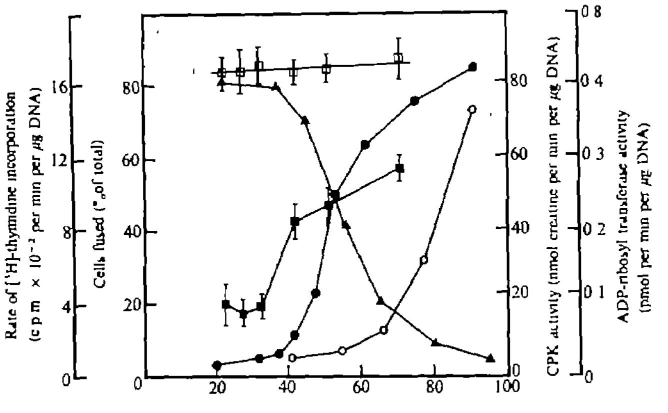

Fig. 1 Muscle cell differentiation *in vitro*. Myoblasts were isolated from the thigh muscle of 12-day-old chick embryos by mechanical dissociation and selective plating according to the method of Mân *et al.*[31]. Cell suspensions were seeded at 0.5×10^6 cells per 6-cm collagen-coated plates in 2.5 ml M199 medium (Earle's salts) supplemented with 10% (v/v) selected horse serum, 2.5% (v/v) embryo extract, 1 mM glutamine, 50 U/ml^{-1} penicillin and 50 µg ml^{-1} streptomycin. Cultures were then incubated, at 37 °C, in 95% air 5% CO_2 and subjected to a complete change of medium at 24 h. The specific substrate of ADPRT is NAD^+, to which intact cell membranes are impermeable. Isolated nuclei are unsuitable because the process of nuclear isolation induces DNA strand breaks and activates ADPRT[9,10]; thus it is necessary to use permeabilized cells to measure ADPRT activity. The myoblast and myotubes were made permeable to ^{3}H-NAD by a mild, brief hypotonic treatment by a modification of the method of Reinhard *et al.*[32]. Muscle cells on 6-cm plates were washed three times with ice-cold Pucks saline A (5.4 mM $MgCl_2$, 137 mM NaCl, 4.2 mM $NaHCO_3$ and 5.5 mM glucose). The plates were then flooded with 1.0 ml of a hypotonic solution containing 9 mM HEPES *p*H 7.8, 5 mM dithiothreitol, 4.5% (w/v) dextran (average molecular weight 110,000), 1 mM EGTA and 4.5 mM $MgCl_2$. The plates were then kept at 4 °C, until more than 90% of the cells had become permeable to Trypan blue; this was routinely achieved after ~20 min for both myoblasts and myotubes; the kinetics were similar in the two sets of cells (data not shown). Permeabilization was monitored by studying plates set up in parallel. After permeabilization, the plates were transferred to a water bath at 25 °C, and the ADP-ribosyl transferase assay was started by the addition of 200 µl of a solution containing 200 mM HEPES *p*H 7.8, 750 mM KCl, 5 mM dithiothreitol, 4.5% (w/v) dextran, 1.35 M sucrose, 7.0 mM EGTA, 4.5 mM $MgCl_2$, 6.0 mM isonicotinic acid hydrazide, 18 mM NaF and 600 µM ^{3}H-NAD (20 mCi mmol^{-1}). The enzyme incubation was continued for 10 min and then terminated by the addition of 2 ml of ice-cold 20% (w/v) trichloroacetic acid (TCA), containing 1% (w/v) nicotinamide and 2% (w/v) tetrasodium pyrophosphate. The acid-insoluble radioactivity was then estimated by scintillation counting. The DNA content of each sample was determined by the method of Kissane and Robins[33]. The acid-insoluble radioactivity was demonstrated to be poly(ADP-ribose)[34]. Incorporation was linear for at least 10 min at all stages of culture studied. The permeabilization procedure induced no detectable DNA strand breaks and at the end of the ADPRT assay the single-strand DNA molecular weight on alkaline sucrose gradients was the same as that in the untreated cells (results not shown). Total ADPRT activity was estimated by including a final concentration of 0.5% (v/v) Triton X-100 and 100 µg ml^{-1} of DNase I in the assay mixture. ■, Physiological ADPRT activity; □, total potential ADPRT activity. The fraction of muscle cells fused (●) was determined as previously described[29]. Each point in the figure is the mean of at least six measurements in three separate experiments; the bars represent the standard error of the mean. Percentage of fused myoblasts (●) and CPK activity (○) were measured as previously described[35]. The rate of DNA synthesis (▲) was estimated by pulsing the cultures with 1 µCi ml^{-1} ^{3}H-thymidine (1 Ci mmol^{-1}) for 1 h.

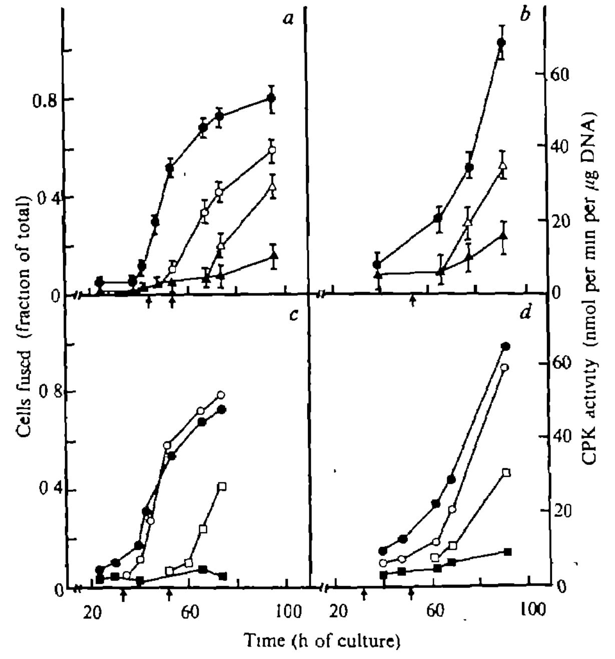

Fig. 2 Reversible inhibition of myoblast terminal differentiation. Reversible inhibition by 3-aminobenzamide (*a*, *b*) and by depletion of NAD levels (*c*, *d*). *a* And *c* show myoblast cell fusion, *b* and *d* show CPK activity. *a*, *b*, Muscle cells were prepared and cultured as described in Fig. 1 legend. ●, Control; ▲, continuous presence of 8 mM 3-aminobenzamide. The medium containing inhibitor was removed and replaced with medium from parallel control cultures of the same age at 44 h (○) or 54 (△) h. Each point is the mean of six measurements in three separate experiments; the bars are the standard errors. CPK activity was estimated as previously described[35]. *c*, *d*, Muscle cells were cultured in nicotinamide-free medium with dialysed serum and embryo extract. ■, No nicotinamide; 1 mM nicotinamide added at the time of plating (0 h ●), at 34(*c*) or 32(*d*) h of culture (○), or at 52 h of culture (□) Each point is the mean of at least two independent experiments. The arrows indicate the time at which the inhibitor was removed or nicotinamide returned in each experiment, as indicated above.

Because of a possible objection to the use of enzyme inhibitors, we also experimentally manipulated ADP-ribosylation by partially depleting the cells of their NAD content, by nicotinamide starvation[13]. After 44 h of culture in a nicotinamide-free medium the NAD level had fallen to 29 ± 3% (mean ± s.e.m., $n = 6$ in two separate experiments). Nicotinamide (1 mM) can restore the control NAD level within 4.5 h. The NAD-depleted myoblasts do not fuse to form multinucleated fibres (Fig. 2*c*), nor do they show the usual rise in CPK activity (Fig. 2*d*). This block in cell differentiation is reversible; the re-addition of 1 mM nicotinamide to the depleted cultures at 32 or 52 h reverses the inhibition 6 or 8 h later respectively (Fig. 2*c*, *d*).

In several cases, in both prokaryotes and eukaryotes, alterations in gene activity involve changes in the DNA, including DNA modification, gene rearrangement and DNA transpositions[23,24]. We might therefore expect DNA strand breaks to appear during certain types of change in gene expression and cytodifferentiation.

In alkaline sucrose gradients which estimate the size of single-stranded DNA, the DNA from proliferating cultures has a high molecular weight (Fig. 3*a*). At 44 h, when only 15% of the cells have fused (see Fig. 1), there is a significant reduction in the molecular weight of the cellular DNA (Fig. 3*b*). By 53 h the maximum number of single-strand breaks are present (Fig. 3*c*), although only 50–60% of the myoblasts have fused. The molecular weight then remains constant up to 92 h of culture (Fig. 3*c*–*f*). Only single-strand breaks appear in the DNA; there is no decrease in the molecular weight of the DNA in neutral sucrose gradients containing ionic detergents between 25 and 92 h of culture (Fig. 3*g*). We have been unable to establish what happens after 92 h of culture, so we do not yet know whether the DNA breaks persist or are subsequently rejoined. The experiments displayed in Fig. 3 are also 'mixing' experiments, and show that in our experimental conditions, mixing of early (without strand breaks) and late (with strand breaks) cells does not induce strand breaks in the DNA of early cells (see Fig. 3*b*–*f*).

Alkaline sucrose gradients detect single-strand breaks that are induced by the alkaline conditions of the gradients (for example, apurinic sites), as well as true single-strand breaks which had been generated enzymatically. We have distinguished between these two phenomena by examining the sedimentation rate of nucleoids[25]. In neutral sucrose gradients containing 1.0 M NaCl, nucleoids from 24 and 38 h cultures sediment rapidly; in contrast, 6 h later, at 44 h, the nucleoids sediment more slowly, and by 68 h a considerable number of metabolically induced single-strand DNA breaks are evident (Fig. 4*a*). Increasing the salt concentration of the gradients to 1.95 M NaCl, and thus stripping the nuclei of yet more of their protein, does not alter the results significantly (Fig. 4*a*). Thus, the reduction in the sedimentation of the nucleoids is not due to changes in the protein composition of the chromatin.

This inference is also drawn from several experiments with ethidium bromide. In the presence of 5 μg ml^{-1} ethidium

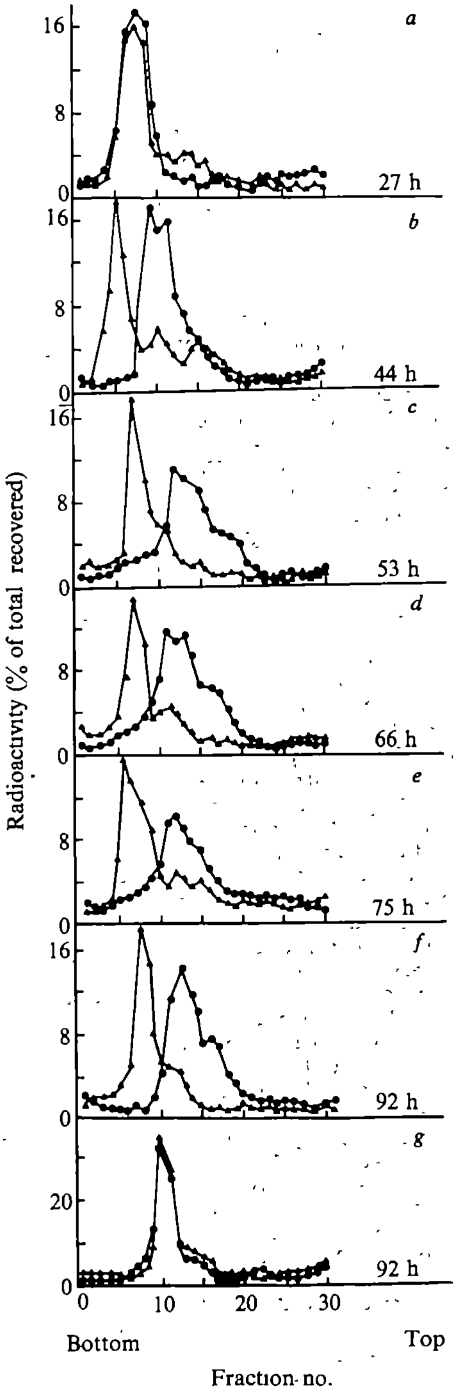

Fig. 3 *a*–*f*, Molecular weight analysis of muscle cell DNA by alkaline sucrose gradients. Muscle cell cultures were radioactively labelled by the continuous presence of 1 μCi ml^{-1} ^{3}H-thymidine, or of 0.2 μCi ml^{-1} ^{14}C-thymidine. Before collecting the cells for the analysis of DNA molecular weight they were incubated in fresh medium for a further 3 h in the absence of radioactive nucleosides. The plates were washed twice in phosphate-buffered saline (PBS) containing 10 mM EDTA, and then cells were scraped off in the PBS-containing EDTA using a rubber policeman. 50 μl of ^{3}H-labelled and 50 μl of the ^{14}C-labelled cell suspensions, each containing the equivalent of $1–2 \times 10^4$ nuclei, were mixed and deposited in 400 μl of lysis buffer (100 mM NaOH, 100 mM NaCl, 10 mM EDTA, 0.2% (w/v) sarcosyl) over a 12-ml, 5–25% linear sucrose gradient containing 100 mM NaOH and 100 mM NaCl. The gradients were kept in the dark for 30 min at 20 °C, and then centrifuged for 2 h at 25,000 r.p.m. in a Beckman SW27 rotor. 400-μl fractions were collected on filter strips from the bottom of the gradients and the TCA-insoluble radioactivity estimated by scintillation counting. ▲, 27-h cultures labelled with ^{14}C-thymidine; ●, 44-, 53-, 66-, 75- and 92-h cultures labelled with ^{3}H-thymidine. g, Molecular weight analysis of muscle cell DNA by neutral sucrose gradients. Muscle cell cultures were radioactively labelled and collected as described above. The equivalent of $1–2 \times 10^4$ nuclei were deposited in 400 μl of lysis buffer (0.2% (w/v) sarcosyl, 0.08% (w/v) sodium deoxycholate, 1.5 M NaCl, 100 mM sodium citrate and 20 mM EDTA, *p*H 8.9) over a 12 ml 5–25% linear sucrose gradient containing 2.0 M NaCl, 10 mM sodium citrate and 1.0 mM EDTA, *p*H 8.9, over a cushion of 0.5 ml 60% sucrose. The gradients were kept in the dark for 1 h at 20 °C, and then centrifuged for 1 h at 20,000 r.p.m. in a Beckman SW27 rotor. 400-μl fractions were collected on filter strips from the bottom of the gradients and the TCA-insoluble radioactivity estimated by scintillation counting. ▲, 25-h culture labelled with ^{14}C-thymidine; ●, 92-h culture labelled with ^{3}H-thymidine.

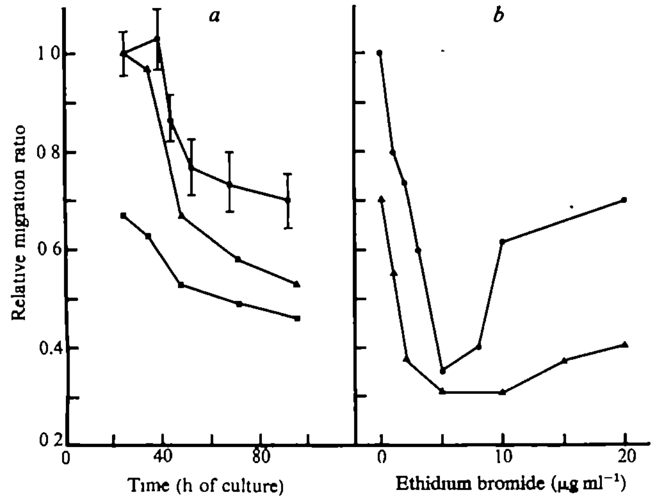

Fig. 4 *a*, The nucleoid sedimentation rate of muscle cells at different stages in culture. 50 μl of muscle cells suspended in PBS (containing the equivalent of $1\text{–}5\times10^5$ nuclei) were deposited inside 150 μl of lysis buffer (giving a final concentration of 2 mM EDTA, 0.5% (v/v). Triton X-100 and 100 mM Tris-(hydroxymethyl)-aminomethane *p*H 8.0) over a 4.6 ml 15–30% linear sucrose gradient containing 1 mM EDTA and 10 mM Tris-(hydroxymethyl)-aminomethane *p*H 8.0. The lysis mixture and the gradients also contained 1.0 M NaCl (●), 1.95 M NaCl (▲), or 1.95 M NaCl and 5 μg ml⁻¹ ethidium bromide (■). The gradients were kept in the dark for 30 min at 20 °C and then centrifuged at 10,000 r.p.m. in a Beckman SW50.1 rotor for 30 min. The gradients were collected from the bottom of the centrifuge tubes and pumped through a flow cell. The positions of the nucleoids were determined by measuring the absorbance at 254 nm, and are expressed as a fraction of the distance migrated by the nucleoids of 24-h cultures in the same conditions (●, ▲) or in 1.95 M NaCl (■). The error bars represent the standard error of the mean of at least three measurements. Nucleoids are residual nuclei stripped of most of their proteins by high-salt solutions and neutral detergents. Single-strand breaks in local domains of supercoiled DNA relieve the supercoiling, and lead to an expansion of the volume of the nucleoids and to a slower rate of sedimentation. *b*, The effect of ethidium bromide on the sedimentation rate of muscle cell nucleoids. Procedure as in *a* except that the lysis mixture and the gradients contained 1.95 M NaCl and the indicated concentration of ethidium bromide. Nucleoids from 24-h (●) or 72-(▲) h cultures were kept in the lysis mixture for only 15 min before being centrifuged at 10,000 r.p.m. for 45 min. The nucleoid sedimentation rate is expressed as a fraction of the distance migrated by nucleoids from 24-h cultures in the absence of ethidium bromide. The binding of intercalating agents, such as ethidium bromide, to DNA molecules under topological constraint produces a characteristic biphasic effect. With increasing concentrations of intercalating agents the number of superhelical turns are reduced and eventually a minimum value is reached. Increasing the concentration further introduces 'negative' turns in the DNA[36]. The introduction of single-strand breaks in the local DNA domains eliminates the topological constraint and reduces the characteristic effect of the intercalating agent on the nucleoid as a whole. These changes in the extent of DNA supercoiling are reflected in the sedimentation rate of the nucleoids in neutral sucrose gradients[37].

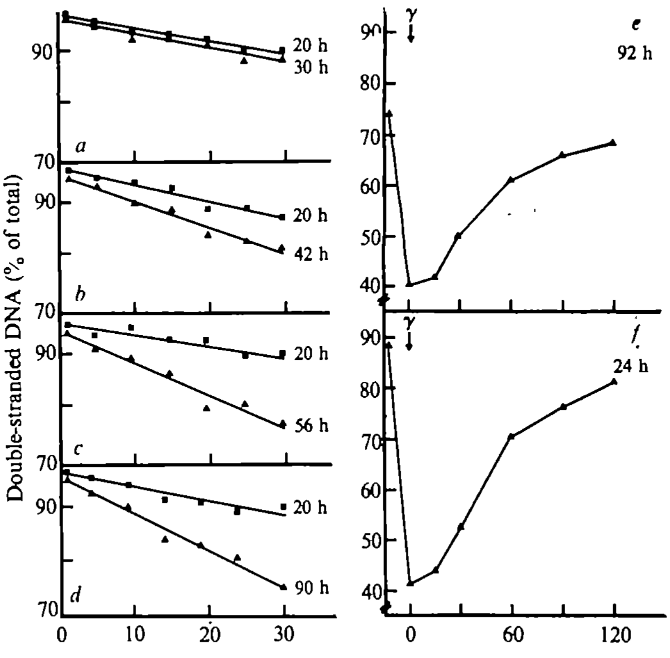

Fig. 5 *a–d*, Rate of DNA strand separation in alkali. The rate of DNA strand separation in alkali was measured with the Rydberg[27] modification of the Ahnström–Erixon alkaline denaturation and hydroxyapatite procedure[26,28]. Briefly, DNA strand separation was initiated by the injection of 50 μl of cell suspension containing the equivalent of $1\text{–}2\times10^4$ nuclei from a 20-h culture labelled with ^{14}C-thymidine (■) and $1\text{–}2\times10^4$ nuclei from ^{3}H-thymidine-labelled cultures at 30, 42, 56 or 90 h of culture (▲) into 1 ml of the alkaline solution containing 30 mM NaOH, 10 mM Na_2HPO_4 and 900 mM NaCl, *p*H 12.0. The injection was forceful to ensure adequate and immediate mixing of the cell suspension and the alkaline solution. This mixture was immediately placed in the dark and kept at 20 °C for the required period. The DNA strand separation was terminated at intervals by the injection of 1 ml of ice-cold 34 mM HCl. Within 5 s of the neutralization, the solution was sonicated for 15 s. Before separating the single- and double-stranded DNA on hydroxyapatite columns, 80 μl of 10% (w/v) SDS solution was added to each sample (~2.0 ml) to obtain a final SDS concentration of 0.4%. Samples were then loaded on 5×30 mm hydroxyapatite columns equilibrated with 10 mM potassium phosphate buffer, *p*H 6.9. The unbound nucleotides were eluted with 2 ml of 10 mM potassium phosphate buffer and the single- and double-stranded DNA was eluted with 3×0.5 ml of 125 mM and 400 mM phosphate buffer respectively. The total radioactivity loaded, and the single- and double-stranded DNA recovered, were measured by scintillation counting. A standard curve was established by exposing 24-h myoblast cultures to 50–300 rad of γ-radiation (results not shown). These cultures have accumulated very few endogenous single-strand breaks at this time. The logarithm of the percentage of double-stranded DNA remaining after 30 min in alkali declines linearly with the applied dose of radiation. Chick muscle cell DNA as a molecular weight of $\sim10^{12}$, calculated from a diploid DNA content of 2.5 pg per nucleus. We estimate that approximately two single-strand breaks are formed per 10^{12} daltons per rad[38]. We can now convert the percentage of double-stranded DNA remaining to the number of single-strand breaks per 10^{12} daltons. *e*, *f*, Rate of DNA repair following γ-radiation. 24- And 92-h muscle cell cultures were irradiated with 300 rad of γ-radiation. The rate of strand-rejoining was estimated by measuring the rate of DNA strand separation in alkali following the re-incubation of the irradiated cells in fresh medium for the indicated times.

bromide, the sedimentation of nucleoids from 24-h cultures is reduced from 1.0 to 0.67 (Fig. 4*a*). However, the same concentration of ethidium bromide has a much smaller effect on the sedimentation at 96 h, which is only reduced from 0.56 to 0.46. In nucleoids from 24-h cultures, increasing concentrations of ethidium bromide induce the biphasic effect characteristic of DNA molecules under topological constraints (Fig. 4*b*), whereas nucleoids from 72-h cultures are affected less (Fig. 4*b*), probably due to the presence of single-strand breaks. We have estimated the number of single-strand DNA breaks[26–28] in differentiating muscle cell DNA, from 20 to 90 h (Fig. 5*a–d*); few single-strand breaks were detectable in the DNA at either 20 or 30 h, but by 42 h approximately 50–100 single-strand breaks per 10^{12} daltons (per diploid genome) had appeared. By 56 h they had increased to 100–300 per genome (Fig. 5*c*), and did not increase thereafter (Fig. 5*d*).

The presence of DNA strand breaks in differentiating cells may be attributed to a general deficiency of DNA repair.

However, these differentiating muscle cells are proficient in repair and can readily rejoin single-strand breaks. There is no detectable decline in the ability to rejoin DNA strand breaks caused by γ-irradiation (Fig. 5*e*, *f*). In the 92-h cultures (Fig. 5*e*), a significant number of DNA strand breaks exist before irradiation; exposure to 300 rads of γ-radiation would be expected to introduce a further 600 breaks, about twice as many as were metabolically engendered. The rate at which these breaks were rejoined was the same as that in 24-h myoblasts—but rejoining stopped when the number of breaks had dropped to the endogenous level. This implies that these endogenous breaks are retained in the face of a proficient DNA strand-rejoining capacity, perhaps because they are of a special character or occur at specific nucleotide sequences; alternatively, there may be a shift in the steady-state level of breaks.

Our observations have been confirmed and extended in subsequent studies showing that ADPRT activity is required for the differentiation of the protozoan *Trypanosoma cruzi* (G. T. Williams, personal communication), the stimulation of resting circulating human lymphocytes[29] and the cytodifferentiation of hepatocytes[30]. The involvement of DNA strand breaks in some categories of cellular differentiation may be the molecular basis for the requirement of ADPRT in cytodifferentiation.

Thus we propose that a reduction in the single-strand molecular weight of the DNA and the consequent obligatory increase in the intrinsic, physiological ADPRT activity are integral and early events in the cellular differentiation of primary chick myoblast cultures. The single-strand breaks may signify some underlying mobility of the genome. The results reported here are consistent with the notion that nuclear ADPRT participates commonly in situations where nicking and rejoining of DNA occur.

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Effects of altered [ADP-ribose]$_n$ metabolism on expression of fetal functions by adult hepatocytes

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The nuclear enzyme ADP-ribosyltransferase (ADPRT) catalyses the postsynthetic modification of chromatin proteins[1]. The significance of [ADP-ribose]$_n$-modified chromatin proteins for the regulation of chromatin function is not understood[1]. Recently, [ADP-ribose]$_n$ biosynthesis has been demonstrated to participate in the repair of some types of DNA damage[2–4]. Here we demonstrate that another function of [ADP-ribose]*n* biosynthesis is related to the regulation of the expression of two fetal functions of cultured hepatocytes—the K-type (III) isozyme of pyruvate kinase, and γ-glutamyltranspeptidase, which is also a marker of early neoplastic transformation of liver cells[5].

An involvement of ADP-ribosylation in cellular differentiation has been inferred from the observation of a temporal relationship between alterations in ADPRT activity and engagement of cells in terminal differentiation[6–11]. However, [ADP-ribose]$_n$ might primarily regulate the proliferative activity[12,13] of cells undergoing terminal differentiation. It has therefore not been possible to ascribe definitively the expression of individual cellular functions during differentiation to changes in ADP-ribosylation activity[1]. The recent introduction of 3-aminobenzamide, the most specific inhibitor of ADPRT known, has made it possible to determine directly the potential involvement of this enzyme in a particular biological process[2,3]. We have used this inhibitor to determine the role of ADPRT in the expression of fetal functions by adult rat hepatocytes in primary monolayer culture.

We found that in rat hepatocytes cultured on collagen gel-nylon meshes[14], ADPRT activity sharply increased to a maximum after 3 days in culture and then decreased to the low levels seen in freshly isolated hepatocytes (Fig. 1*a*). Coincident with the decrease in ADPRT activity, the activities of γ-glutamyltranspeptidase and the fetal (K-type) isozyme of pyruvate kinase sharply increased (Fig. 1*b*). These two enzyme activities are normally high in fetal hepatocytes *in vivo* but disappear shortly after birth[15,16]. Previous studies in cultured hepatocytes have clearly shown that the expression of fetal pyruvate kinase isozyme activity is the result of induction of the isozyme protein[16]. We observed that induction of the fetal pyruvate kinase isozyme was totally inhibited in hepatocyte cultures treated with 3-aminobenzamide (10 mM) or nicotinamide (25 mM), two inhibitors of ADPRT (Fig. 2); this indicates that induction of the isozyme and alterations in ADP-ribosylation activity (Fig. 1) are causally related. The specificity of action[3] of the ADPRT inhibitors was indicated by the inability of the non-inhibitory analogue[2], 3-aminobenzoic acid (1–10 mM), to prevent the expression of the fetal pyruvate kinase isozyme (Fig. 2*a*). In addition, the dose–response curves and the apparent K_is for the actions of 3-aminobenzamide and nicotinamide on ADPRT and pyruvate kinase in intact hepatocytes were very similar[4]. When hepatocytes were maintained for 2–7 days in medium containing 10 mM aminobenzamide or 25 mM nicotinamide, a 70–95% inhibition of ADPRT activity was observed. This inhibition was reversible,

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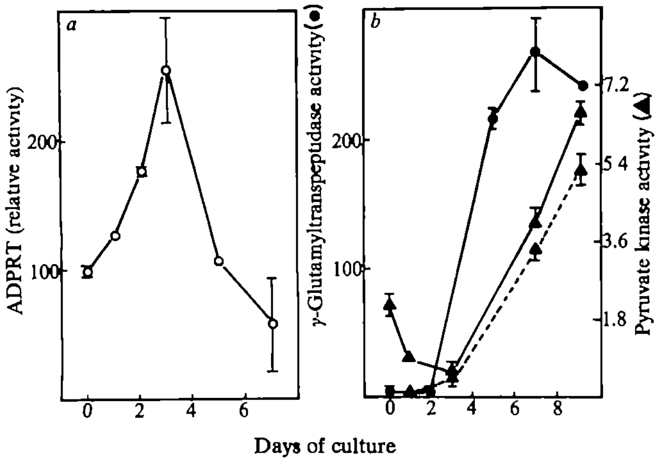

Fig. 1 Alterations in the activities of ADPRT, γ-glutamyltranspeptidase and pyruvate kinase of adult rat hepatocytes cultured on collagen gel-nylon meshes. *a*, Hepatocytes were isolated from male Holtzman rats (200–250 g, fed *ad libitum*) and cultured on collagen gel–nylon meshes as described previously[14] At the times indicated, hepatocytes were recovered from the substratum by brief collagenase treatment[14] and ADPRT activity measured as described elsewhere[4] using ^{3}H-NAD as substrate in cells made permeable with digitonin[23]. All activities are expressed relative to the activity of freshly isolated hepatocytes. Separate experiments indicated that the ADPRT activities measured as ^{3}H-NAD incorporation into hepatocytes following gentle permeabilization with digitonin[23] reflect the physiological enzyme activity of intact cells Thus, a much higher proportion of [2,8-^{3}H]adenosine radioactivity added to the medium of intact hepatocytes was found to be covalently linked to chromatin proteins after a labelling period (3-h pulse) that coincided with high ADPRT activity (day 3 of culture) (Fig. 1) compared to an identical labelling period immediately after plating of cells. This incorporation of [2,3-^{3}H]adenosine radioactivity was inhibited in hepatocytes cultured in the presence of 3-aminobenzamide (10 mM) or nicotinamide (25 mM). *b*, Activities of γ-glutamyltranspeptidase (●—●) and pyruvate kinase (▲—▲) were determined as described[14,16]. ▲---▲, Fetal (K-type) isozyme activity of pyruvate kinase. γ-Glutamyltranspeptidase activity is expressed as nmol *p*-nitroaniline formed per μg DNA per h[14] and pyruvate kinase activity reflects the change in absorbance at 340 nm per μg DNA per h[16]. Bars indicate mean ± s.d of 3–5 measurements in separate cell batches; symbols without bars represent the averaged values from two determinations in two separate cell batches.

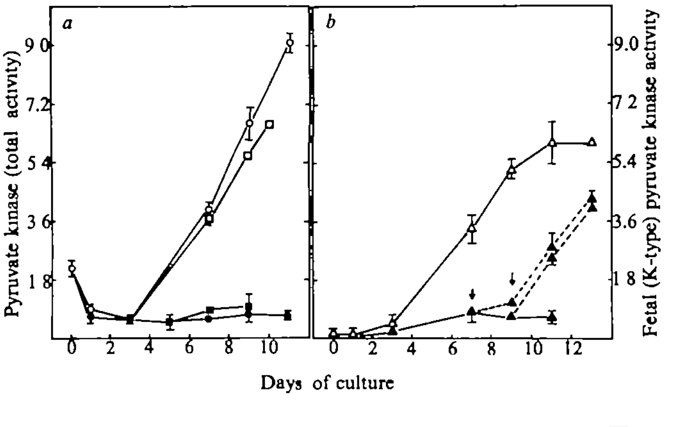

Fig. 2 Effects of the ADPRT inhibitors, 3-aminobenzamide (■, 10 mM) nicotinamide (●, 25 mM), and the non-inhibitory analogue 3-aminobenzoic acid (□, 10 mM), on the expression of pyruvate kinase activity by hepatocytes cultured on collagen gel–nylon meshes. *a*, Total pyruvate kinase activity of hepatocytes maintained in the presence of the above and in control cultures (○). *b*, Activity of the fetal (K-type) isozyme of pyruvate kinase in untreated control (△—△) and nicotinamide-treated (▲—▲) hepatocyte cultures. ▲---▲, Fetal isozyme activity after withdrawal (arrows) of nicotinamide from the culture medium. Bars indicate mean ± s.d. of 3–5 separate cell prepartions; symbols without bars represent the averaged values from two determinations in two separate cell batches. None of the ADPRT inhibitors had any direct effect on pyruvate kinase activity *in vitro*.

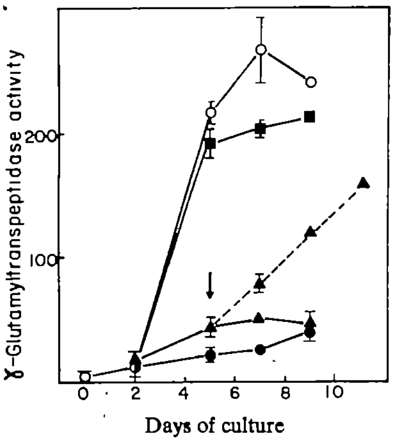

Fig. 3 Effect of nicotinamide (▲—▲, 25 mM), 3-aminobenzamide (●—●, 10 mM) and 3-aminobenzoic acid (■—■, 10 mM) on the expression of γ-glutamyltranspeptidase activity by hepatocytes cultured on collagen gel–nylon meshes. ○—○, Enzyme activities of untreated control cultures. ▲---▲, Enzyme activity after withdrawal (arrow) of nicotinamide from the culture medium. Inhibition of γ-glutamyltranspeptidase expression was also observed with other ADP-ribosylation inhibitors such as isonicotinamide, benzamide and theophylline[4]. Results expressed as mean ± s.d. of 3–5 separate measurements; symbols without bars represent the averaged values from two determinations in two separate cell batches. None of the ADPRT inhibitors had any direct effect on γ-glutamyltranspeptidase activity *in vitro*.

as was the repression of the fetal pyruvate kinase isozyme (Fig. 2*b*).

We then investigated the possibility that ADP-ribosylation might have a coordinative function in the expression of hepatocellular functions *in vitro*. We studied the effect of 3-aminobenzamide and nicotinamide on the expression in cultured hepatocytes of albumin, α-fetoprotein and γ-glutamyltranspeptidase[14]. The expression of γ-glutamyltranspeptidase activity was suppressed by this treatment (Fig. 3), whereas the expression of α-fetoprotein and albumin was unaffected (data not shown) as judged by immunological quantification of these proteins using monospecific antibodies[14]. The inhibition of γ-glutamyltranspeptidase expression required the presence of ADP-ribosylation inhibitors during the first 5 days of culture. Treatment of hepatocytes after day 5 of culture, that is, when ADPRT activity had returned to low levels (Fig. 1), caused only marginal inhibition (~10%) of this expression. The specificity of action for ADPRT inhibitors was indicated by the inability of the non-inhibitory analogue[2], 3-aminobenzoic acid (1–10 mM), to inhibit the expression of γ-glutamyltranspeptidase activity (Fig. 3). A histochemical study[17] revealed that γ-glutamyltranspeptidase was uniformly suppressed in all hepatocytes of a culture dish, indicating that the actions of these ADPRT inhibitors were not selective for a small cell population within the liver (data not shown). In addition, the effect of these inhibitors on γ-glutamyltranspeptidase expression was reversible (Fig. 3).

Nicotinamide has been reported to inhibit general protein synthesis during the first 24 h of hepatocyte culture [18]. Although we could confirm this observation, we found that both nicotinamide (25 mM) and 3-aminobenzamide (10 mM), stimulated ^{3}H-leucine incorporation into total hepatocellular proteins after this initial culture period. Therefore, we conclude that the effects of these two ADPRT inhibitors on the expression of pyruvate kinase and γ-glutamyltranspeptidase are not due to a general inhibition of protein synthesis. Similarly, previous studies from our laboratory demonstrated the independence of the expression of fetal functions by cultured hepatocytes from DNA synthesis activity[14], which increases after 2–3 days in culture in the absence of measurable cell replication. Although this increase in DNA synthesis was slightly inhibited by nicotinamide (25 mM), continuous treatment of hepatocyte cultures for up to 9 days with 3-aminobenzamide did not affect this activity (data not shown). Thus, the action of ADPRT inhibitors on pyruvate kinase expression by the hepatocytes was not related to effects on DNA synthesis. These data also suggest that the presumed specificity of some ADPRT inhibitors might not be absolute. Similarly, effects on extranuclear ADP-ribosylation reactions cannot be totally excluded.

There is increasing evidence that ADPRT activity is largely dormant in the cell nucleus unless factors inducing DNA fragmentation are manifest[1,19–21]. In general, DNA fragmentation presumably then initiates the activation of ADPRT. To test this, we examined hepatocellular DNA for the presence of DNA strand breaks in relation to changes in ADP-ribosylation. Using a slight modification[4] of the sensitive technique of Birnboim and Jevcak[22], we were unable to find an increase in the number of DNA strand breaks either before or concomitant with changes in ADPRT activity (Fig. 1) although we observed a gradual but slow increase in the number of DNA strand breaks with increasing time in culture. This increase was unaffected by 3-aminobenzamide or nicotinamide (data not shown). These results suggest that the transient and spontaneous alterations in ADPRT activity seen in primary cultures of hepatocytes (Fig. 1) are not causally related to DNA fragmentation, which agrees with the observation of Jackowski and Kun[6] who found age-dependent variations in the ADPRT activity of cardiocyte nuclei in the absence of measurable DNA fragmentation.

Our results show that [ADP-ribose]$_n$ metabolism is involved in the regulation of the expression of two fetal functions by adult hepatocytes in primary culture. It will be interesting to determine whether carcinogen-induced alterations in cellular [ADP-ribose]$_n$ metabolism[1] are related to some of the well known functional phenotypic changes associated with neoplastic transformation[5,15].

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Role of DNA breaks and ADP-ribosyl transferase activity in eukaryotic differentiation demonstrated in human lymphocytes

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ADP–ribosyl transferase (ADPRT) is a DNA-dependent nuclear enzyme which covalently attaches ADP–ribose moieties derived from NAD to proteins to form mono- or poly-ADP–ribosyl derivatives[1,2]. ADPRT activity is strongly stimulated by breaks in DNA[3,4] and the enzyme is required for efficient DNA repair[5–7]. Several reports have suggested that the metabolism of poly- or mono-(ADP–ribose) might also be involved in differentiation in various eukaryotes[1,2,8–12]. In the most convincing of these, inhibitors of ADPRT were shown to block the differentiation of chick myoblasts *in vitro*[11,12], and differentiation was accompanied by the appearance of single-strand breaks in DNA. We present here evidence that ADPRT activity is obligatory early in the mitogen-induced activation of human peripheral blood lymphocytes. The requirement for this enzyme coincides with a rapid (1–8 h) rejoining of single-strand breaks present in the DNA of quiescent lymphocytes. Hence, DNA breaking and rejoining, regulated by ADPRT, may be involved in a general mechanism of differentiation.

Quiescent lymphocytes can be stimulated to differentiate by various polyclonal activators, or 'mitogens', which mimic the effect of specific antigens on individual cells[13–15]. This differentiation involves a change in morphology, initiation of proliferation and acquisition of various effector functions. In the present study human peripheral blood lymphocytes, comprising ~70% T cells, were activated by three mitogens: pokeweed mitogen (PWM), concanavalin A (Con A) and *Phaseolus vulgaris* phytohaemagglutinin (PHA)[13].

The effect of several inhibitors of ADPRT, that is, benzamide[16], 3-aminobenzamide[17], 3-methoxybenzamide[17] and 5-methylnicotinamide[18], on the mitogen-stimulated incorporation of ^{3}H-thymidine was studied. If they were added slightly before or at the same time as the mitogen, all of the inhibitors significantly decreased the response to all three mitogens; 3-methyoxybenzamide and 5-methylnicotinamide were the most effective (Fig. 1). Conversely, if the inhibitors were added to the cells 24 h after PHA or Con A, before any DNA synthesis had occurred, a substantial response was observed (Fig. 1*b*, *c*). The incorporation of ^{3}H-thymidine at day 2 was near to normal and only the cells responding more slowly and synthesizing DNA at days 3 and 4 were substantially affected by this later addition of inhibitors.

At the concentrations used, the inhibitors did not affect lymphocyte viability, assessed by exclusion of eosin, over a period of 7 days in culture. Similarly, they did not inhibit the lymphoid cell cycle in the absence of differentiation as evidenced by their lack of significant effect on the proliferation in culture of previously stimulated lymphoblasts (Fig. 1*b*, *c*), human T lymphoblastoid cell lines Molt 4 and CEM (unpublished observations) and murine leukaemic cells L1210[5].

To define more precisely the time during their response at which the cells are susceptible to inhibition, lymphocytes were stimulated with PHA or Con A, and 3-methoxybenzamide or 5-methylnicotinamide was added to the cultures at various times. Figure 2 shows the response observed at day 2. Within 1 h of PHA stimulation, a significant proportion (~20%) of the cells synthesizing DNA at day 2 had escaped from the inhibitable stage and by 12–16 h a plateau was reached when 60–70% of cells had passed through the phase at which they were susceptible to inhibition. The time of addition of inhibitors which corresponded to a response at day 2 of 50% of normal was 15.1±2.3 h for Con A and 8.0±4.2 h for PHA. This is

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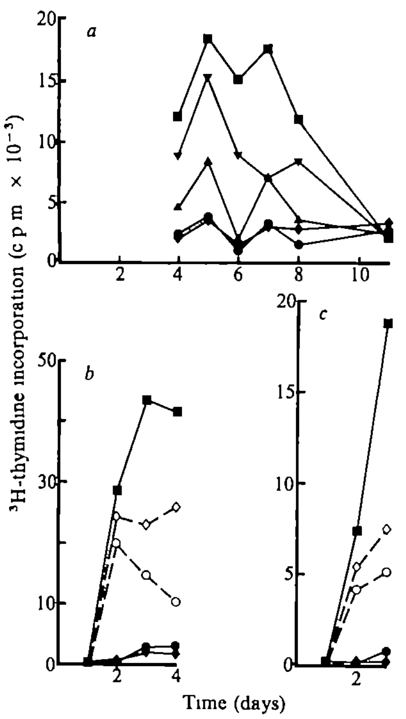

Fig. 1 Effect of inhibitors of ADPRT on lymphocyte transformation. Mononuclear leukocytes, comprising 95% lymphocytes, were islolated from human peripheral blood[28] and incubated at 10^6 cells ml^{-1} at 37 °C in an atmosphere of 5% CO_2 in RPMI 1640 medium containing 25% human AB serum and 1/250 dilution of PWM (Gibco) (*a*), 5% fetal calf serum and 2.5 $\mu g\ ml^{-1}$ PHA leukoagglutinin (Pharmacia) (*b*), or 5% fetal calf serum and 1 $\mu g\ ml^{-1}$ Con A (Sigma) (*c*). The cells were incubated with medium containing no inhibitor (—■—), 2.5 mM benzamide (—▼—), 5 mM 3-aminobenzamide (—▲—), 2.5 mM 3-methoxybenzamide (—◆—) or 5 mM 5-methylnicotinamide (—●—) from 30 min before the addition of mitogen. Alternatively, freshly dissolved 2.5 mM 3-methoxybenzamide (--◇--) or 5 mM 5-methylnicotinamide (--○--) was added to the cultures 24 h after addition of mitogen. [6-^{3}H]thymidine (5 Ci $mmol^{-1}$; Amersham) was added to a final concentration of 2 $\mu Ci\ ml^{-1}$ for the final 6 h before the cells were collected onto Whatman GF/C paper and washed with water using a cell harvester at the times indicated after mitogen stimulation. Each point represents the mean c.p.m. incorporated into DNA of duplicate 200-µl cultures. Control cultures without mitogen incorporated 300–1,000 c.p.m. The time course of the PWM response is invariably slower than that of the other two mitogens.

consistent with the slower response to Con A compared with PHA in our system (Fig. 1).

ADPRT is involved in increasing DNA ligase II activity[7] and so the depression of the lymphocyte response by inhibitors of ADPRT suggests that ligation of DNA might be an obligatory early event during lymphocyte activation. To test for this, the migration of lymphocyte 'nucleoids' on sucrose density gradients was observed at various times after mitogen stimulation (Fig. 3). Nucleoids are prepared from eukaryotic cells by lysis in a non-ionic detergent in the presence of high salt and EDTA[19,20]. They have lost most of the nuclear protein and consist of DNA in a supercoiled conformation. Any breaks in DNA decrease the constraints on the supercoiled structure, allowing it to expand and causing the nucleoid to migrate more slowly in the density gradient. Determination of the nucleoid sedimentation rate is therefore a very sensitive method for detecting single-strand breaks[21,22].

Nucleoids from PHA-stimulated lymphocytes sedimented faster through the density gradients than those derived from quiescent lymphocytes. This difference could be detected as early as 1 h after stimulation and was maximal after about 8 h (Fig. 3). The change in sedimentation rate was much slower if 3-methoxybenzamide or 5-methylnicotinamide was included in the culture medium (Fig. 3, compare *e* with *f* in expt 1; compare *c* with *h*, and *e* with *i* in expt 2). The increased rate of sedimentation of nucleoids from activated lymphocytes demonstrated that single-strand breaks present in the DNA of resting lymphocytes are rapidly rejoined after PHA stimulation. This early ligation of DNA breaks may be directly involved in inducing the expression of new genes responsible for the programmed activation of lymphocytes. Other workers have reported that lymphocyte nucleoids sediment much more slowly than those from most other cells[19,20,22], but the existence of DNA breaks in quiescent lymphocytes was unexpected and intriguing.

There is a close temporal correlation during lymphocyte activation between DNA strand rejoining and susceptibility to blocking of the response by ADPRT inhibitors (compare Figs 2 and 3). This observation, together with the inhibition of strand rejoining by inhibitors of ADPRT (Fig. 3) and the established role of ADPRT in DNA repair[5–7,21], suggests that this enzyme controls the rejoining of DNA strand breaks early in differentiation.

Recently, ADPRT activity has been demonstrated in membranes and implicated, via adenylate cyclase, in the hormonal regulation of certain tissues[23]. It is conceivable that the chemicals we have used produce their effects by inhibiting the membrane enzyme rather than the nuclear enzyme. However, as mitogen stimulation of lymphocytes, unlike the effect of some polypeptide hormones, cannot be attributed simply to adenylate cyclase activity[13], we consider this possibility unlikely.

ADPRT activity and the formation of single-strand breaks in DNA have previously been correlated with differentiation in chick embryo myoblasts[11,12]. In addition, the morphological differentiation of the protozoan parasite, *Trypanosoma cruzi*, can be blocked by inhibitors of ADPRT (manuscript in preparation). Most importantly, the enzyme activity does not seem to be involved in cell cycle processes because its inhibition does not affect proliferation in the absence of differentiation in any of the three systems. This illustrates the operational specificity of the inhibitors. The concordant results obtained in these diverse systems support the hypothesis that ADPRT and transient DNA breaks are widely involved in the differentiation of eukaryotic cells.

It is possible that these events are involved in a specific rearrangement of genetic material as a ubiquitous mechanism for coherently altering gene expression during differentiation. Indeed, recent data suggest that there are some differences in

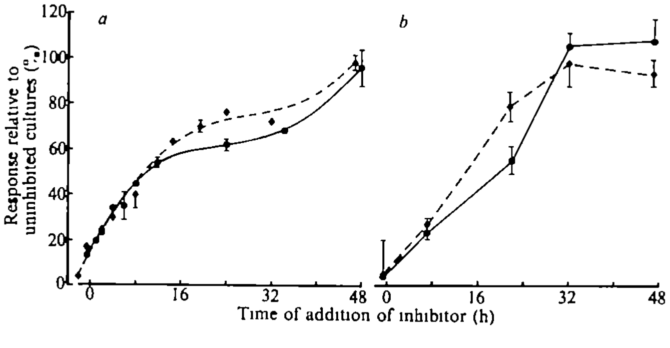

Fig. 2 Kinetics of susceptibility to inhibition during lymphocyte activation. In four separate experiments, lymphocytes were isolated from four individuals and cultured with PHA (*a*) or Con A (*b*) at time zero as described for Fig. 1. 2.5 mM 3-methoxybenzamide (—◆—) or 5 mM 5-methylnicotinamide (—●—) was added to the cultures at the times indicated before or after addition of mitogen. The cultures were pulsed with ^{3}H-thymidine at 48 h and collected at 52 h as described for Fig. 1. The results are expressed as a percentage of the response (c.p.m. incorporated into DNA) in control cultures to which medium was added at the same time as inhibitor solution was added to the test cultures; 100% response represents 25,000–35,000 c.p.m. for PHA and 5,000–10,000 c.p.m. for Con A. Each point represents the mean ± s.e.m. for triplicate 200-µl cultures.

the arrangement of genetic material between different tissues of an individual person[24]. The controlled appearance of temporary breaks in the DNA backbone could also be required to relax the tightly packed chromatin structure in particular areas, thus allowing the transcription of new genes[25-27].

In addition to the exciting theoretical concepts outlined above, our present report suggests that chemicals which inhibit ADPRT activity may have several practical clinical uses. For example, if mitogen-induced activation of lymphocytes *in vitro* is indeed a valid model for antigenic stimulation of the immune system *in vivo*, the inhibitors should specifically block new immune responses while allowing established ones to continue. This would be very useful for many applications requiring immunomodulation, in particular during tissue transplantation.

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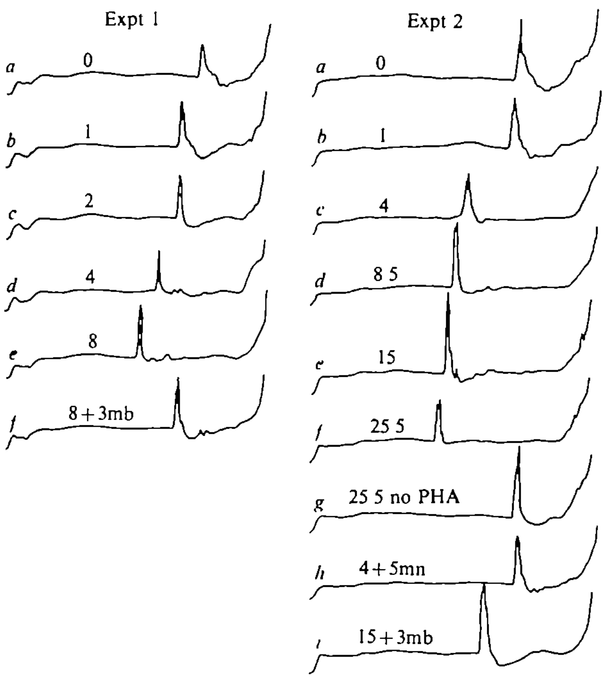

Fig. 3 Changes in nucleoid sedimentation rate during lymphocyte activation. In two separate experiments, lymphocytes were isolated from two individuals and 5×10^5 cells cultured with PHA as described for Fig. 1*b*. After varying times, the cells were washed once with ice-cold 10 mM sodium phosphate, 0.15 M NaCl *p*H 7.3 and deposited (in ~100 µl) inside a layer of 400 µl of 0.5% Triton X-100, 10 mM Tris, 10 mM EDTA, 2 M NaCl *p*H 8.0 lying on top of a 11.5 ml gradient of 5–20% (w/v) sucrose in 10 mM Tris, 10 mM EDTA, 2 M NaCl *p*H 8.0 in a Beckman SW41 centrifuge tube. After incubation for 15 min at 20 °C, the gradients were centrifuged at 25,000 r.p.m. ($100{,}000g_{max}$) for 30 min at 20 °C and eluted from the bottom through the flow cell of a Unicam SP8000 recording spectrophotometer set at 260 nm. The absorbance profiles (bottom of gradient on the left) obtained from the following cultures are presented. Expt 1: lymphocytes incubated at 37 °C with PHA for: *a*, 0; *b*, 1 h; *c*, 2 h; *d*, 4 h; 8 h (*e*, *f*). Before addition of mitogen, cells were incubated for 16 h at room temperature in medium alone (*e*) or 2.5 mM 3-methoxybenzamide (*f*). Expt 2: lymphocytes incubated at 37 °C for: *a*, 0; *b*, 1 h; *c*, 4 h; *d*, 8.5 h; *e*, 15 h; *f*, 25.5 h; *h*, 4 h; *i*, 15 h with PHA or for 25.5 h without PHA(*g*). Before addition of mitogen, cells were incubated for 4 h at 37 °C in medium alone (*c* and *e*) or 5 mM 5-methylnicotinamide (*h*) or 2.5 mM 3-methoxybenzamide (*i*). Each nucleoid does not sediment totally independently and so the peaks obtained represent the average migration of a heterogeneous population.

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A new wild-derived *H-2* haplotype enhancing *K-IA* recombination

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It is generally accepted that the overall recombination frequency between the *H-2K* and *H-2D* loci of the mouse major histocompatibility complex (*H-2*) is at a low level of about 0.4%[1]. However, the value fluctuates depending upon the combinations of *H-2* haplotypes in the heterozygote[1]. We re-examined the intra-*H-2* recombination frequency, using three B10.W congeneic strains which carry the *H-2* complex of Japanese wild mice. Of the three, one strain, B10.MOL-SGR($H\text{-}2^{wm7}$), showed an extremely high incidence of recombination (about 3%) in all combinations with three different inbred B10 congeneic strains. The serological typing of these recombinants revealed that most of the crossing-over points had occurred between the *H-2K* and *IA* loci. These results suggest that the *wm7* haplotype enhances specific recombination irrespective of *H-2* partners in heterozygous parents.

In the past five years we have produced several new *H-2* congeneic strains by introducing the *H-2* complex of Japanese wild mice into the C57BL/10J (B10) genetic background[2]. Japanese wild mice, *Mus musculus molossinus*, are genetically very remote from the European subspecies, *Mus musculus domesticus*. It is estimated that ancestral mice diverged into these subspecies about one million years ago[3,4]. Consequently, many genetic mutations seem to have accumulated in their genomes. Recent genetic research has shown that most inbred laboratory strains are derived from the European subspecies[5]. This finding prompted us to re-examine the intra-*H-2* recombination frequency in heterozygotes between inbred B10 congeneic strains and the B10.W strains produced in our laboratory.

All mice used in the present study are shown in Table 1, together with serological typing of their *H-2K*, *IA*, *IE*, *Ss-Slp* and *H-2D* loci. Table 2 summarizes the recombination

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Table 1 Immunological characters of *H–2* complex in the mouse strains used

		Presence of antigenic specificities expressed by cytotoxic titres												
		K		IA			IE		S		D			
Strain	*H–2* haplotype	K.23	K.33	Ia.2	Ia.18	Ia.20	Ia.7	(IE^k)	Ss	slp	D.4	D.32	D.2	Origin of *H–2* complex
B10.A	*a*	80	0	>160	>160	0	40	80	h	a	>160	0	0	Inbred, A/WySn
B10.BR	*k*	80	0	>160	>160	0	40	80	l	o	0	>160	0	Inbred, C57BR/cd
B10	*b*	0	>160	0	0	>160	0	0	h	o	0	0	>160	Inbred, C57BL/10J
B10.MOL-TEN1	*wm1*	0	0	0	0	0	0	0	l	o	0	0	0	Jap. wild (Sapporo)
B10.MOL–SGR	*wm7*	0	0	0	0	0	40	0	h	o	0	0	0	Jap. wild (Fukuoka)
B10.MOL–YNG	*wm11*	0	0	0	0	0	0	0	h	o	0	0	0	Jap. wild (Yonaguni Is.)

H–2 alloantisera were produced in our laboratory according to the immunization schedules described in the NIH catalogue[6]. These are D23(anti-K 23), D33(anti-K.33), D4(anti-D.4), D32(anti-D.32) and D2(anti-D.2). Monoclonal anti-Ia antibodies were kindly given by Dr K. Ozato of NIH. These are 26-7-11S(anti-Ia.2), 26-8-16S(anti-Ia.18), 25-9-35(anti-Ia.20), 14-4-S(anti-Ia.7) and 17-3-3S(anti-IE^k). For the last one, the antigenic specificity detected is unidentified. Ss-Slp typing was performed by Ouchterlony double diffusion analysis using rabbit anti-Ss serum and Slp alloantiserum which were kindly given by Dr S. Sakai of Kanazawa University. The microcytotoxicity test was carried out using the flat-type titration plate method previously described[7]. Titres of H–2 antisera and Ia antibodies are indicated as the maximum dilution which kills more than 90% and 50% of the cells respectively.

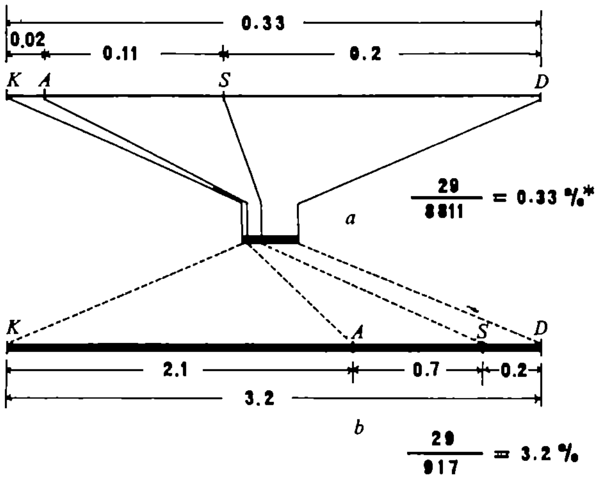

Fig. 1 Comparison between two genetic maps of *H–2* complex. *a*, Standard genetic map previously reported[1]. The map distances represent the average recombination frequency (%) in various combinations of *H–2* haplotypes for males and females together. *b*, Map constructed from the data shown in Table 3. The distances between each loci are slightly underestimated, because two recombinants, *aw16* and *kw1*, whose crossing-over origins were unidentified, were excluded from the calculation of distances*. Data from ref. 1.

frequencies estimated from the present experiments. We screened 1,510 mice and detected 30 recombinants. The overall recombination frequency was estimated to be 1.9%, but the values were strikingly different among the combinations of *H–2* haplotypes. Of the five combinations, *a/wm1* and *a/wm11* showed the standard frequency as reported previously by many investigators. On the other hand, the frequency in the heterozygotes between *wm7* and three different inbred *H–2* haplotypes, *a*, *k* and *b*, was 3–4%, which is almost one order of magnitude higher than the normal recombination frequency.

In order to define the positions of crossing-over in the above three *H–2* combinations, the genotypes of the *IA*, *IE* and *Slp* loci were determined by serological typing. Table 3 demonstrates two features of the recombination event involving the *wm7* haplotype. First, the crossing-over positions are concentrated in the chromosomal segment between *H–2K* and *IA* loci, a segment which many laboratories have observed to have the lowest incidence of recombination (0.02%)[1]. This is conspicuous in the *k/wm7* and *b/wm7* heterozygotes. Of the 27 recombinants examined, at least 19 are derived from crossing-over in this segment. It should be noted that the average recombination frequency among the three combinations is 2.1%. On the other hand, crossing-over between the *Slp* and *D* loci is rare. Crossing-over between these segments was found in only two recombinants, although this is the site with the highest incidence of crossing over that has been observed in combinations of inbred *H–2* haplotypes so far. This site-specific enhancement of recombination can be clearly demonstrated by comparing the genetic map constructed from the present data with the standard map. Figure 1 shows that the relative distance between each marker locus becomes larger toward *H–2K* end in the present map, compared with the standard map. Second, reciprocal recombinant types do not seem to occur with equal frequency. In the eight *b/wm7* heterozygotes, only K^bD^w recombinants were detected except for *bw7*. This tendency is also shown in the *a/wm7* heterozygote, although the K^wD^a type is dominant in this case.

Some of these recombinants were crossed with a third party mouse and the offspring serologically typed with *H–2* antisera. All recombinants examined were confirmed by the test (Table 3). Furthermore, fourteen recombinants derived from the *a/wm7* heterozygote have been established as *H–2* recombinant strains and are now maintained by sib-mating. These were again serologically typed, this time using anti-*H–2*^{wm7} antiserum. For this purpose, (B10.BR×DBA/2J) anti-B10.MOL-SGR alloantiserum was produced and subsequently absorbed by B10.A(R201) (*H–2*^{aw1}, $K^kA^wE^wS^wD^w$) and B10.A(R217) (*H–2*^{aw17}, $K^wA^wE^wS^dD^d$) respectively. It is expected that the resultant two antisera will detect either *H–2D* or *H–2K* specific antigens of the *wm7* haplotype. The reaction of these two antisera to the established recombinant strains was consistent with that expected from the genotypes determined by the antisera detecting the inbred *H–2* haplotypes (Table 3), indicating that genetic rearrangement had occurred in these strains.

This is the first report describing a genetic factor that enhances intra-*H–2* recombination. The mechanism by which the B10.MOL-SGR strain raises the recombination frequency in combinations with other strains is, at present, not clear. However, as all the mice used in the present cross experiment carry the common genetic background of the B10 mouse, the

Table 2 Frequency of intra-*H–2* recombination

H–2 haplotype of heterozygous parents	Method of screening	No. of mice screened	No. of recombinants	Recombination frequency (%)
a/wm1	HA*	294	1	0.3
a/wm11	HA	299	0	—
a/wm7	HA	539	16	3.0
k/wm7	CT†	174	5	2.9
b/wm7	CT	204	8	3.9
Total		1,510	30	1.9

To detect the recombinants, the females of F_1 hybrids between inbred B10 congenic strains and three B10.W strains were backcrossed to the respective parental B10.W strains. The offspring were screened with two H–2 alloantisera which direct either the H–2K or H–2D private antigens carried by the parental inbred *H–2* haplotypes. For the typing, haemagglutination test was used if the antiserum was suitable, if not microcytotoxicity tests against inguinal lymph node cells were carried out. * Haemagglutination test; † cytotoxicity test.

Table 3 Genetic composition of the recombinant *H-2* haplotypes

Parental H-2 haplotype	Established strain	Recombinant H-2 haplotype	Origin of *H-2* subregions					Progeny test	Cytotoxic titres anti-H-2^{wm7} adsorbed with	
			K	*A*	*E*	*S*	*D*		R201	R217
a/wm7	B10.A(R201)	*aw1*	*k*	│ *w**	*w*	*w*	*w*	Yes†	0	4
	B10.A(R202)	*aw2*	*k*	*k*	*k*	*d*	│ *w*	Yes	0	4
	B10.A(R203)	*aw3*	*k*	*k*	*k*	│ *w*	*w*	Yes	0	4
	B10.A(R204)	*aw4*	*w*	│ *k*	*k*	*d*	*d*	Yes	64	0
	B10.A(R205)	*aw5*	*w*	*w*	*w*	│ *d*	*d*	Yes	64	0
	B10.A(R206)	*aw6*	*w*	│ *k*	*k*	*d*	*d*	Yes	64	0
	B10.A(R207)	*aw7*	*w*	│ *k*	*k*	*d*	*d*	Yes	64	0
	B10.A(R208)	*aw8*	*k*	*k*	*k*	*d*	│ *w*	Yes	0	4
	B10.A(R209)	*aw9*	*w*	│ *k*	*k*	*d*	*d*	Yes	64	0
	B10 A(R211)	*aw11*	*k*	*k*	*k*	│ *w*	*w*	Yes	0	4
	B10 A(R212)	*aw12*	*w*	*w*	*w*	│ *d*	*d*	Yes	64	0
	B10.A(R213)	*aw13*	*w*	*w*	*w*	│ *d*	*d*	Yes	32	0
	B10.A(R214)	*aw14*	*w*	│ *k*	*k*	*d*	*d*	Yes	64	0
		aw15	*w*	│ *k*	*k*	*d*	*d*	NT‡	NT	NT
		aw16	*k*	?	?	?	*w*	NT	NT	NT
	B10.A(R217)	*aw17*	*w*	*w*	*w*	│ *d*	*d*	Yes	64	0
k/wm7		*kw1*	*k*	?	?	?	*w*	NT		
		kw2	*w*	│ *k*	*k*	?	*k*	Yes		
		kw3	*w*	│ *k*	*k*	?	*k*	Yes		
		kw4	*w*	│ *k*	*k*	?	*k*	Yes		
		kw5	*k*	│ *w*	*w*	?	*w*	Yes		
b/wm7		*bw1*	*b*	│ *w*	*w*	?	*w*	Yes		
		bw2	*b*	│ *w*	?	?	*w*	NT		
		bw3	*b*	│ *w*	*w*	?	*w*	Yes		
		bw4	*b*	│ *w*	?	?	*w*	NT		
		bw5	*b*	│ *w*	*w*	?	*w*	Yes		
		bw6	*b*	│ *w*	*w*	?	*w*	Yes		
		bw7	*w*	│ *b*	*b*	?	*b*	Yes		
		bw8	*b*	│ *w*	*w*	?	*w*	Yes		

Antisera and antibodies used to define the origin of *H-2* subregions are shown in Table 1 The S region is serologically typed with Slp alloantiserum. Solid vertical bars indicate the crossing-over positions

* The letter *w* indicates wild-derived subregions. † Tested. ‡ Not tested.

difference in the recombination frequency seems to be a direct result of the *H-2* complex. It seems likely that the chromosomal structure of the *H-2K* end, especially between *H-2K* and *IA*, of the *wm7* haplotype is different from that of other haplotypes. The ultimate interpretation should be made through investigations on the DNA level.

Generally, the intra-*H-2* recombination frequency is higher in the female than in the male. The total frequency in the female was 0.32%, and in the male, 0.19%, based on the progeny-tested recombinants[1]. Therefore, the frequency of female recombination estimated in the present experiment is still one order greater than the normal value. We are now proceeding to evaluate male recombination frequency in heterozygotes, between the *wm7* and several other *H-2* haplotypes.

The B10.MOL-SGR strain will provide a powerful tool for investigating the structure and functions of *H-2* genes, because we can obtain the recombinant derived from *K*-end specific crossing-over by screening at most 30–40 mice, probably irrespective of *H-2* haplotypes. Moreover, the resultant recombinants would be useful in detecting unknown Class I and Class II loci if they are located at the *H-2K* end, especially between the *H-2K* and *IA* loci.

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Allelic polymorphism and complexity of the genes for HLA-DR *β*-chains—direct analysis by DNA–DNA hybridization

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HLA-DR antigens are highly polymorphic cell surface glycoproteins involved in the control of the immune response. They are important in allograft rejection and in their linkage to certain disease susceptibilities. The allelic polymorphism of HLA-DR antigens is carried by the β-chain. We have isolated cDNA clones encoding several distinct HLA-DR β chains and other β-chains from related loci, including DC, making it possible to analyse this complex genetic system directly by Southern hybridization and to demonstrate the polymorphism of these DNA sequences in the human genome. Hybridization patterns obtained with DNA of one DR-heterozygous and three DR-homozygous cell lines suggest the presence of a number of different genes for these two β-chain loci. Extensive polymorphism of the restriction pattern was detected in individuals of different DR specificities, with each of the three restriction enzymes used. Analysis of HLA-DR directly at the DNA level may lead to a simpler typing procedure and may eventually refine the specificities so far determined either serologically or by cellular assay.

The I region of the mouse major histocompatibility complex (H-2) controls the immune response[1]. These genes encode polymorphic glycoproteins (Ia antigens) expressed mainly at the cell surface of macrophages and lymphocytes and involve

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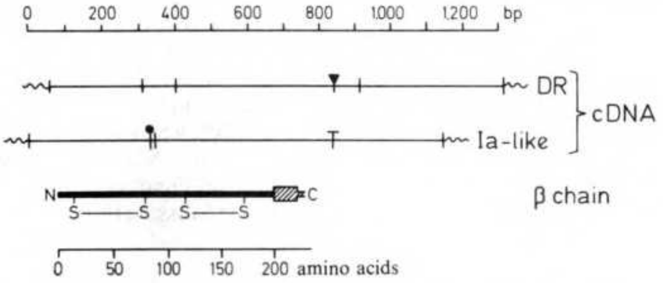
0 200 400 600 800 1,000 1,200 bp
DR
Ia-like
cDNA
N
C
S S S S
β chain
0 50 100 150 200 amino acids

Fig. 1 Maps of cDNA clones encoding two distinct human Ia antigen β-chains. Clones were isolated as described elsewhere[14]. Clone DR β − 1 encodes a β-chain of HLA-DR antigen. Clone Ia-like β-1 encodes a β-chain of another related antigen, probably DC. The sites for several restriction endonucleases are indicated by the following symbols: *Pst*I (|), *Hin*dIII (▼), *Bam*HI (⫯), and *Eco*RI (T). Wavy lines represent pBR322 sequences. The diagram of the β-chain structure is based on the sequence of a human Ia antigen β-chain determined by Kratzin *et al.*[23]. The transmembrane region, shown by a hatched box, and the cytoplasmic region have not been sequenced.

cellular interactions essential for the immune response. The equivalent antigens in humans are coded for by the HLA-D region of the major histocompatibility complex[2,3]. The best known, HLA-DR antigens, consist of an invariant heavy or α-chain[2] and a highly polymorphic light or β-chain[4,6]. Monoclonal antibodies have distinguished subsets of DR antigens[7]. Typing of the polymorphic DR specificities, important for successful organ transplantation[8], is done by serological tests and by the mixed lymphocyte reaction[9]. In addition, there is significant linkage between some HLA-DR specificities and susceptibility to certain diseases[10]. Besides DR, other human Ia antigens have been identified serologically. Their genes (DC) are in close linkage disequilibrium with the classical polymorphic DR locus[11,12]. A third locus, termed SB, has been shown to encode polymorphic Ia antigens[13].

We have cloned cDNA sequences encoding β-chains of HLA-DR antigens and other related Ia-like antigens[14]. Initial studies of these clones, and of other recombinant clones containing corresponding DNA sequences from genomic DNA, strongly suggest the existence of several distinct genes for the HLA-DR β chain and for related Ia-like antigen β chain (data not shown). The availability of these β-chain cDNA clones makes possible a direct analysis of this polymorphic genetic system at the level of the DNA. Using the cloned β-chain sequences as probes we have analysed digested human DNA transferred to nitrocellulose filters by the method of Southern[15]. First, determining the number of different DNA fragments hybridizing to the β-chain probes will yield information about the complexity of these loci. Second, a comparison of the DNA from individuals with different HLA-DR specificities will quantify the allelic polymorphism of the system.

Restriction enzyme maps of the two β-chain sequences used as hybridization probes are shown in Fig. 1. Both cDNA clones were derived from the lymphoblastoid cell line Raji, which is heterozygous at the DR locus (DR 3, w6). HLA-DR β-1 encodes the DR β chain[14]. Ia-like β-1 encodes the β-chain of another human Ia antigen and has been shown to correspond in the mouse to the I-A β chain gene of the I region[16]. Since structural studies have now established a correlation between the α-chains of mouse I-A and human DC[17], we can tentatively consider the 'Ia-like' β-1 clone as specific of DC β chain. DNA from one DR-heterozygous and three DR-homozygous human lymphoblastoid cell lines were analysed: Raji (DR 3, w6), HHK (DR w6, w6), IBW9 (DR 7, 7), and LG2 (DR 1, 1).

The results of hybridizing the two β probes to the four genomic DNAs are shown in Fig. 2. A fairly complex pattern is evident. Depending on the cell line and the restriction endonuclease used, 4–13 bands hybridize with the HLA-DR

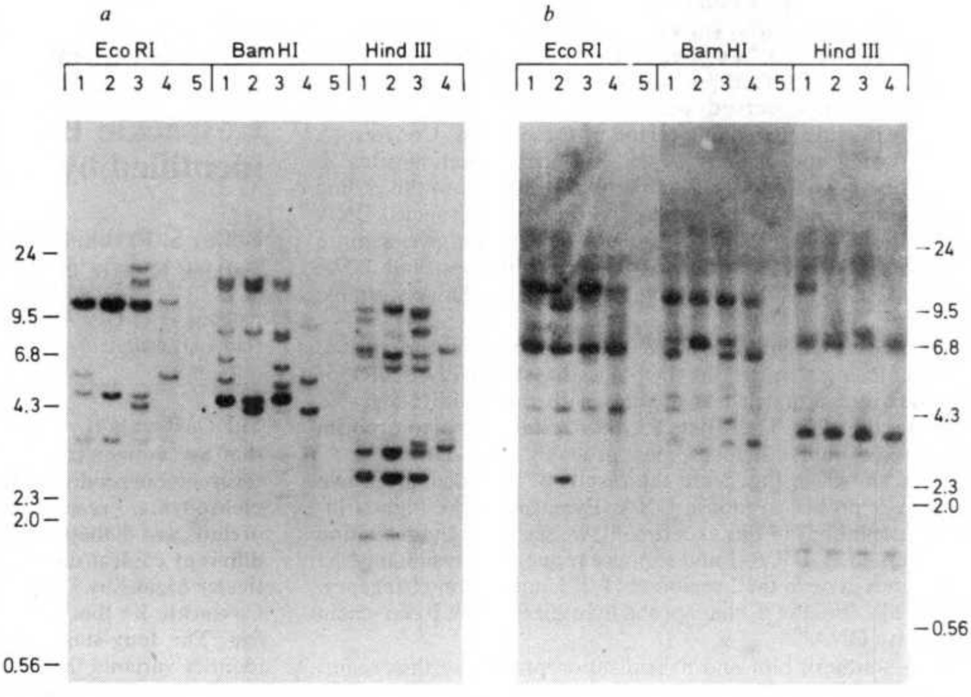

Fig. 2 Hybridization of cDNA sequences of the β-chains of HLA-DR and another Ia-like locus to human and mouse DNA. ^{32}P-labelled probes were purified inserts from cDNA clones HLA-DR β-1 (panel *a*) or Ia-like β-1 (panel *b*). DNA samples were from: 1, human B cell line Raji (DR 3, w6); 2, human B cell line HHK (DR w6, w6); 3, human B cell line IBW9 (DR 7, 7); 4, human B cell line LG2 (DR 1, 1); and 5, BALB/c mouse embryo (H–2^d). Size markers are given in kilobases. High molecular weight DNA was isolated as described elsewhere[19]. Restriction endonuclease digestions were done at 37 °C with *Eco*RI (Boehringer Mannheim), *Bam*HI or *Hin*dIII (Bethesda Research Laboratories) using standard buffer conditions with 1 unit enzyme μg^{-1} for 4 h, at which time another 1 unit enzyme μg^{-1} was added and incubated overnight. Reactions were stopped with EDTA, extracted once with chloroform/isoamyl alcohol (24 : 1), and ethanol-precipitated. Pellets were resuspended in 10 mM Tris-HCl, *p*H 7.6, 1 mM EDTA, 0.1% SDS, 0.05% bromophenol blue, 0.05% xylene cyanol, and 5% glycerol for 4 h at 37 °C. Samples were heated for 5 min at 65 °C just before loading on to 0.6% agarose gels in 200 mM glycine, 15 mM NaOH, *p*H 8.3. About 7 μg of LG2 DNA and 9 μg of the other DNA samples were loaded. Gels were run at 60–100 V for 12 h, treated and transferred to 0.2 μm nitrocellulose filters (Schleicher and Schuell) as described[24]. After transfer, the filters were rinsed in 4 × SSC (SSC = 150 mM NaCl, 15 mM trisodium citrate), then baked for 2 h at 80 °C in a vacuum oven. Filters were prewashed in 5 × SSC, 5 × Denhardt's solution for 1–2 h at 65 °C with gentle shaking, then prehybridized for 2 h at 65 °C in 1 × Denhardt's, 0.75 M NaCl, 5 mM EDTA, 50 mM sodium phosphate buffer, *p*H 7.0, 10% dextran sulphate, 0.1% SDS, and 50 μg ml^{-1} sonicated denatured herring DNA. Hybridization was for 8–12 h at 65 °C in the same buffer as used for prehybridization with 1×10^6 c.p.m. ml^{-1} of ^{32}P-labelled DNA ($>10^8$ c.p.m. μg^{-1}). Filters were washed at 65 °C for 30 min twice with each of the following solutions: 5 × SSC, 1 × Denhardt's, 0.1% SDS, 0.1% sodium pyrophosphate; 2 × SSC, 0.1% SDS; 0.5 × SSC; 0.1 × SSC.

β-1 clone and 3-9 bands hybridize with the Ia-like β-1 clone. This multiplicity of bands contrasts markedly with the simple pattern obtained when the cDNA clone encoding the non-polymorphic HLA-DR α-chain is used as probe[18,19]. However, the degree of complexity of the HLA-DR β chain sequences observed here contrasts also with the more extensive complexity found in class I genes of both man[20] and mouse[21,22], where up to 40 DNA fragments hybridize to the specific HLA or H-2 probes.

The finding of multiple hybridizing bands is a strong indication of the existence of several genes for the β-chains of both HLA-DR and the Ia-like (or DC) loci. This is in complete agreement with our recent finding in genomic and cDNA clones from homozygous cell lines of several distinct genes for both the HLA-DR β-chain and the β-chain of the DC locus, as well as several additional genes for other related Ia-like β chains, not yet identified (data not shown). Some of the weakly hybridizing bands could result from sequence differences between the probe (isolated from Raji cells) and the homologous sequences in other cell lines. The existence of strongly cross-hybridizing bands in each of the cell lines indicates highly conserved regions in the β-chain gene. In addition, weak signals can also result from DNA fragments containing only a short region of homology.

The genetic polymorphism of the HLA-DR locus, which previously could be demonstrated and assayed only at the level of the expressed proteins, is detected here directly at the level of the DNA. The four cells lines with different DR specificities generated different patterns of hybridizing bands with all three restriction endonucleases tested (Fig. 2*a*). For example, with *Bam*HI digestion, only one weak band is shared by all four cell lines out of their 4–11 bands. Only a few of these 4–11 fragments are unique to any one cell line. This analysis of genomic DNA can detect not only polymorphic endonuclease restriction sites located within the coding region, and corresponding to polymorphic changes seen in the antigens, but also possible polymorphic sites located within non-coding introns or in sequences flanking the DR genes. The *Eco*RI, *Hin*dIII and *Bam*HI restriction sites present in the coding regions will probably not account for all of the observed polymorphism. This indicates that the polymorphism observed with an HLA-DR β-chain probe extends outside the coding region of these genes. Conversely, by choosing specific probes and specific restriction sites, it should be possible to focus and limit the analysis to the coding region concerned in the polymorphism of the antigens. DNA from the DR-heterozygous line Raji does not give a more complex pattern than the homozygous cell lines, and DNA from a HLA-DR 1,1 homozygous shows a relatively simple pattern.

Significant polymorphism is also observed at the related Ia-like β locus when the DNA is digested with *Eco*RI or *Bam*HI, but is much less obvious with the *Hin*dIII-digested samples (Fig. 2*b*). These data therefore establish polymorphism at another human Ia locus, most probably HLA-DC.

Also shown in Fig. 2 are the results of hybridizing the two human β probes to mouse DNA. Even under the high stringency conditions of this experiment, we see cross-hybridization between HLA-DR β-1 and a mouse sequence, presumably the analogous gene in the I region of H-2. Under lower stringency, the Ia-like (or DC) β-chain probe hybridizes to the I-A β-chain in mouse DNA[16].

The Southern blot and hybridization procedure thus seems to be a simple and precise technique for detecting HLA-DR allelic polymorphism directly at the DNA level. A more extensive investigation would be necessary to correlate the DR specificities determined classically by serological and cellular tests with the hybridization pattern of the DNA. Such a study would test the feasibility and reliability of 'DNA typing' for DR specificity. In addition, Southern blot analyses might reveal subtle differences in the DNA patterns which could allow a further subdivision of the known DR specificities. Increased precision and large-scale availability of HLA-DR typing would be important because of the association between particular HLA-DR specificities and susceptibility to certain diseases.

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High frequency of antigenic variants among naturally occurring human Coxsackie B4 virus isolates identified by monoclonal antibodies

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The Coxsackie B virus group consists of six serotypes (B1–B6) that are antigenically distinct. In patients, the Coxsackie B4 serotype can produce a variety of clinical diseases (for example, pleurodynia, respiratory illness, meningitis, myocarditis, orchitis and diabetes)[1,2]. It is not known, however, whether the different clinical diseases are due to chance infection of particular organs by Coxsackie B4 or due to antigenic variants of Coxsackie B4 that are inherently different in their tissue tropism. The long-standing reference hyperimmune sera do not identify variants that may exist within each of the serotypes[3]. In an attempt to identify variants of the Coxsackie B4 serotype, we prepared a panel of 18 monoclonal antibodies that neutralized the prototype Coxsackie B4 strain. These monoclonal antibodies have been characterized as to their subclass, neutralization titres and reactivity patterns with recent clinical isolates. Here we have identified over 13 variants of Coxsackie B4 virus and have shown that there are major antigenic differences among naturally occurring isolates. Using monoclonal antibodies as a selecting agent, we find that the frequency of antigenic mutation may be as high as 10^{-4}.

Antibodies	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Isolates of Coxsackie B4															
HI*																
86-3																
204-4																
183-1																▨
286-1					▨		▨									
287-3										▨						▨
225-1										▨				▨		▨
9-4											▨	▨	▨	▨	▨	▨
204-3					▨	▨	▨	▨	▨						▨	
356-1											▨	▨	▨	▨	▨	▨
187-1										▨	▨	▨	▨	▨	▨	▨
38-1								▨	▨		▨	▨	▨	▨	▨	▨
215-2					▨				▨	▨	▨	▨	▨	▨	▨	▨
339-1				▨		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
337-3			▨	▨				▨	▨	▨	▨	▨	▨	▨	▨	▨
302-1		▨	▨	▨		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
128-2		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
208-2		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
288-3		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
59-3	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
17-1	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨

Fig. 1 Neutralization of clinical isolates by monoclonal antibodies. 100 $TCID_{50}$ of each of the 15 clinical isolates (numbered 2 to 16) and the prototype virus (1) were incubated with each of the monoclonal antibodies and the hyperimmune sera (HI) in a microneutralization assay (see legend to Table 1). The entire experiment was repeated twice with comparable results. Open boxes indicate complete neutralization of virus; hatched boxes indicate that the virus was not neutralized.

Hybridomas which secrete monoclonal antibodies specific for Coxsackie B4 virus were produced by fusing $P_3$653 Ag8 myeloma cells with spleen cells from BALB/c mice that were injected five times intraperitoneally with 10^7 plaque-forming units (PFU) of purified prototype Coxsackie B4 virus (JVB strain) obtained from the American Type Culture Collection (ATCC VR-184). The fusion protocol and selection of clones were as described previously[4,5]. Initially, the hybridomas were selected by screening the culture supernatants for virus-specific antibody in a microneutralization assay against the prototype virus. Cultures that were positive for antibody were cloned using the end point dilution method[6]. Clones grown from a single cell were further tested in a radioimmunoassay (RIA) for the production of antibody.

More than 70 monoclonal antibodies arising from 22 hybridomas that neutralize the prototype Coxsackie B4 virus have been obtained. The properties of 18 of these monoclonal antibodies are shown in Table 1. The monoclonal antibodies were predominantly of the IgG class and exhibited different neutralization titres against the prototype virus and precipitated different amounts of virus by RIA. For example, antibody 225-1 had a neutralization titre of 1,536 but bound only 10.8% of the virus in RIA. In contrast, antibody 38-1 had a neutralization titre of only 96, but bound 100% of the virus in RIA. These differences between RIA and neutralization could be due either to varying capacities of the antibody subclasses to bind to the *Staphylococcus aureus* cells used in the RIA[7] or to different concentrations of various epitopes required for neutralization. Monoclonal antibody 59-3 failed to neutralize the prototype virus and together with 17-1 served as a control. Most of the hybridomas have been grown in BALB/c mice[8], and ascites fluid with neutralization titres greater than 1:130,000 have been obtained. The monoclonal antibodies in Table 1 were also tested by neutralization against other serotypes of the Coxsackie B virus group (B1, B2, B3, B5 and B6) and found to be non-reactive.

The 18 neutralizing antibodies described in Table 1 were used for the antigenic analysis of 15 fresh clinical isolates obtained from two different State Health Departments. Each isolate was passaged three times or less in culture. Although all the isolates were neutralized by type-specific (anti-B4) hyperimmune reference serum, the monoclonal antibodies show that there is considerable antigenic variation among the Coxsackie B4 isolates (Fig. 1). Some of the monoclonal antibodies (that is 86-3, 204-4) neutralized all the isolates, whereas others (128-2, 208-2, 288-3) neutralized none of the isolates. Figure 1 also shows that the monoclonal antibodies recognized 15 different epitopes. The clinical isolates differed from the prototype virus in 2–12 of the 15 different epitopes. Some of the isolates (for example, 11 to 13) appear to be antigenically identical, others differ from each other at only one epitope (for example, 2 and 3; 6 and 7; 8 and 9), and still others differ from each by as many as 10 epitopes (for example, 2 and 16). Analysis of the data revealed 13 different antigenic variants. Although there were some exceptions (for example, 286-1), the monoclonal antibodies that gave the highest neutralization titres against prototype virus (Table 1), usually neutralized the greatest number of clinical isolates (Fig. 1). One possible explanation for such a broad specificity is that the epitopes with which these monoclonal antibodies are reacting are present in high concentration on the surface of prototype virus and are broadly expressed among the clinical isolates.

The identification of a large number of naturally occurring clinical variants by monoclonal antibodies suggested that the rate of mutation of Coxsackie B4 virus might be high. To test

Table 1 Reactivity of monoclonal antibodies with prototype Coxsackie B4 virus

Antibodies	Subclass	RIA (% bound)	Neutralization titre against prototype virus
Hyperimmune serum	–	100.0	1,024
86-3	IgG2b	42.0	4,056
204-4	IgG2a	75.9	192
183-1	IgG1	100.0	768
286-1	IgG2b	91.9	12
287-3	IgG2a	82.2	1,536
225-1	IgG2b	10.8	1,536
9-4	IgG3	51.5	384
204-3	IgG*	88.1	24
356-1	IgG2a	69.3	96
187-1	IgG2a	87.8	48
38-1	IgG2a	100.0	96
215-2	IgG2a	92.3	48
339-1	IgG2a	86.9	48
337-3	IgG2a	58.9	24
302-1	–†	97.1	12
128-2	IgM	61.9	6
208-2	IgG2b	7.2	2
288-3	–†	13.0	6
59-3	–†	27.0	<2
17-1	–†	0.9	<2

Prototype virus and clinical isolates were grown in LLC-MK2 (MK) cells to a maximum titre of 2×10^6 $TCID_{50}$ ml^{-1} (Tissue Culture Infective Dose calculated by the Reed–Muench method[17]) These viruses were diluted to contain 100 $TCID_{50}$ in 0.1 ml before being used in the microneutralization test. The monoclonal antibodies were from tissue culture supernatants. The subclass was determined by double diffusion using rabbit anti-mouse IgG1, IgG2a, IgG2b, IgG3 antisera (Miles Laboratories), and goat anti-mouse IgG (heavy chain) and IgM (heavy chain) (Cappel Laboratories). For the RIA, monoclonal antibodies (50 µl) were mixed with ^{3}H-leucine-labelled purified Coxsackie B4 virus[16] (1,500 d.p.m.) in 150 µl of buffer (0.01 M Tris, 0.1 M NaCl, 0.001 M EDTA, 1 mg ml^{-1} bovine serum albumin and 0.01% Triton X-100). After overnight incubation at room temperature, the immune complexes were precipitated using heat-killed, formalin-fixed *S. aureus* cells[7]. Both the supernatant and the precipitate were counted in a liquid scintillation counter and the per cent radiolabelled virus bound by antibody was calculated To determine the neutralization titre, 100 $TCID_{50}$ of virus in 50 µl were added to wells of a microtitre plate containing 50 µl of threefold serially diluted monoclonal antibodies These were mixed and incubated at 37 °C for 1 h and then 10^4 MK cells in 0.1 ml of growth medium were added to each well. The plates were examined for virus-induced cytopathology 7 days later. All samples were tested in duplicate in two separate experiments with similar results Titres are expressed as the reciprocal of the highest dilution of antibody preventing cytopathology

* Subclass not determined.

† No precipitin line noted.

Table 2 Frequency of antigenic variants in plaque-purified Coxsackie B4 viruses

	None*	Monoclonal antibodies							
		86-4		102-2		183-5		204-4	
Virus	Titre	Titre	Freq.	Titre	Freq.	Titre	Freq.	Titre	Freq
1-1	6.76	1.60	−5.16	1.48	−5.28	1.79	−4.97	2.00	−4.76
5-2	6.78	1.00	−5.78	ND		ND		1.90	−4.88
9-2	6.58	1.78	−4.80	1.30	−5.28	1.00	−5.58	2 57	−4.01
14-2	6.48	1 84	−4.64	1.00	−5.48	ND		1.78	−4.70
15-4	6.98	2.78	−4.20	2.48	−4.50	2.04	−4.94	2.28	−4.70

The prototype virus (1-1) and four different clinical isolates (5-2, 9-2, 14-2 and 15-4) were plaque-purified twice in MK cells with agarose overlay. The plaque-purified viruses were grown up in MK cell monolayers and virus titres determined. Serial 10-fold dilutions of the virus were incubated at 37 °C for 1 h in the absence or presence of undiluted monoclonal antibodies (ascites fluid with titres >1·130,000). The virus–antibody mixtures were then added to monolayers of MK cells in 60 mm Petri dishes and adsorbed for 90 min. The inoculum was removed and the cultures were overlaid with medium containing the same antibodies used for neutralization plus 5% methylcellulose. This was done to prevent any residual virus from reinfecting cells After 7 days, cells were stained with neutral red and the number of plaques were counted and the virus titre determined (expressed in $\log_{10}$). The frequency of mutation was determined by dividing the virus titre in the absence of antibody by the virus titre in the presence of antibody. ND, no plaques observed due to complete neutralization of virus even at the highest concentration of virus inoculum.

* Ascites fluid from a hybridoma that does not produce antibody against Coxsackie B4 virus.

this, prototype virus and four plaque-purified clinical isolates were selected and incubated with four different monoclonal antibodies. Table 2 shows that virus escaping neutralization was found in 17 of the 20 combinations, and, based on the titre of the residual virus, the frequency of mutation was calculated to be $10^{-4.0}$ to $10^{-5.8}$. To ensure that the residual virus represented true variants, plaques were selected randomly from each of the 17 combinations, grown up and re-tested in the absence or presence of the selecting monoclonal antibody. In each case, the selecting monoclonal antibody failed to neutralize the virus, indicating that true antibody-resistant variants had been obtained (data not shown).

In the present study, 18 of 70 antibodies were characterized and their interaction with 15 fresh clinical isolates revealed that the Coxsackie B4 serotype consists of many antigenic variants. Further experiments with another 36 clinical isolates revealed additional antigenic variants (unpublished data). Previously, Coxsackie B4 variants went undetected because polyclonal reference sera were used for typing. The demonstration of a large number of naturally occurring variants suggests that the different clinical pictures seen in Coxsackie B4 infection might be due to variants having different tissue tropism. There are, in fact, specific receptors for picornaviruses on the surface of cells[9–11]. If the antigenic determinants in question are involved in virus attachment to the cell receptor, this could affect the binding affinity of the virus and influence the tropism of the virus for various tissues. Studies are now underway to characterize the antigenic pattern of a large number of Coxsackie B4 isolates obtained from patients with different clinical diseases. If a correlation between antigenic variation and disease pattern could be established, then monoclonal antibodies might prove useful in classifying subtypes of Coxsackie B4 virus. Furthermore, these monoclonal antibodies may be of use in epidemiological studies of antigenic variants of Coxsackie B4 virus obtained in different years and from different geographical locations.

Our studies with monoclonal antibodies as a selecting agent show that Coxsackie B4 mutates at a high rate and that the frequency of antigenic variants may be as great as 10^{-4}. This is rather higher than that reported for influenza virus[12] but is within the range of several estimates for other RNA viruses[13–15]. The high rate of mutation may account for the surprisingly large number of antigenic variants at Coxsackie B4 found in the human population. The fact that some of the variants differed from prototype virus by as many as 12 epitopes suggests that major antigenic changes may periodically develop and thus account for both the persistence of the virus in nature and the different clinical syndromes.

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Synthesis *in vitro* of an exceptionally long RNA transcript promoted by an *Alu*I sequence

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More than 300,000 copies of the ~300 base pair (bp)-long *Alu*I DNA sequence are dispersed throughout the human genome[1–7]. Several *Alu*I sequences have been shown to contain sequences which promote transcription *in vitro* by the enzyme RNA polymerase III[8–11]. While the function(s) of transcripts synthesized from such promoters is unclear, one notion is that RNA polymerase III transcription from an *Alu*I promoter might have an effect on expression of a nearby RNA polymerase II gene, specifically, the β-globin gene (see Fig. 1). However, we found that this *Alu*I promoter is expressed efficiently *in vitro* only in conditions dramatically different from those required for the β-globin and other promoters (both RNA polymerases II and III). Surprisingly, the major transcript produced from this promoter is 2.3 kilobases (kb) long, much longer than any RNA polymerase III transcript described previously.

The DNA structure in the region around the β-globin gene is shown in Fig. 1. The β-globin promoter has previously been shown to be active in the whole-cell lysate transcription system used here[12], although transcription from the *Alu*I promoters was not detected. However, transcription from the distal *Alu*I repeat was observed by one group in a system containing only RNA polymerase III activity[9].

The plasmid pHB8 cleaved with *Eco*RI was used as a template for *in vitro* transcription in an experiment in which the amount of lysate added to reaction mixtures was varied from 60 to 25% of the total reaction volume (Fig. 2*a–d*). At high lysate concentrations, the predominant transcript is a 1.4-kb run-off RNA which arises from RNA polymerase II transcription originating from the β-globin promoter[12]. As the amount of lysate in the reaction mixture is reduced, this RNA is no longer detected. However, a very abundant RNA, ~510 nucleotides long, is now observed (Fig. 2*c*, *d*). As shown below, this RNA results from initiation by RNA polymerase III at the β-globin-distal *Alu*I repeat sequence. (Initiation from the other *Alu*I repeat has not been detected.)

The specific requirements for efficient transcription by RNA polymerase III observed with this promoter are unusual, and not, for example, common to all genes transcribed by this polymerase. Figure 2*e*–*h* shows the results obtained when a cloned fragment of adenovirus serotype 2 (Ad2) DNA containing both an RNA polymerase II and III promoter was transcribed in the same set of conditions used with the globin DNA above. While the RNA polymerase II promoter shows the same response to variations in the amount of lysate added as does the β-globin promoter, the RNA polymerase III promoter is used efficiently at all lysate concentrations tested, with a maximum observed at about 50% lysate (Fig. 2*f*). We have tested one other RNA polymerase III promoter (rat tRNA Leu, obtained from D. Rodi and B. Haas), and found that this promoter responds to variations in lysate concentration in the same manner as the Ad2 RNA polymerase III promoter (results not shown).

When the concentration of lysate is changed, several parameters are simultaneously varied. To determine which of these are responsible for the decrease in transcription of the 510-nucleotide RNA at high lysate concentrations, the following experiment was done. Transcription was first carried out exactly as shown in Fig. 2*d* (that is, 24% lysate). When the concentration of lysate buffer was increased so as to equal the amount present in reaction mixtures containing 60% extract, no specific transcription was detected (results not shown). Therefore, it seems unlikely that the high protein concentration in the reaction mixtures containing 60% extract inhibits transcription. Furthermore, increasing the buffer concentration did not result in synthesis of the 1.4-kb β-globin RNA, indicating that a high protein concentration is required for transcription initiation from this promoter, as has been observed for other RNA polymerase II promoters[13]. The concentration of each buffer component was then varied independently. Increasing $MgCl_2$ from 2.4 to 6.0 mM resulted in an approximately sixfold inhibition; increasing KCl from 24 to 60 mM inhibited transcription threefold, and an increase in glycerol concentration from 4 to 10% caused a 1.5-fold inhibition. The basis for this response is unclear, although an effect on the double-helical structure of the DNA template is a possibility. A similar effect on transcription of an *Escherichia coli* tRNA gene by *E. coli* RNA polymerase has been observed previously[14]. Whether such stringent ionic conditions are required to obtain efficient transcription *in vitro* from any (or all) other *Alu*I promoters is also unknown.

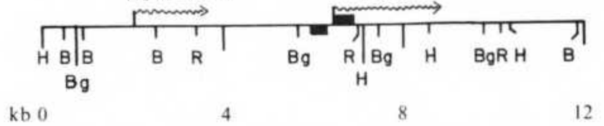

Fig. 1 Map of the human β-globin gene and surrounding sequences. Two inverted *Alu*I repeat sequences are indicated by black boxes. The β-globin primary transcript is indicated by a wavy line, as is the *Alu*I-generated transcript described here. Sizes are in kb. Relevant restriction enzyme sites are: H, *Hin*dIII; B, *Bam*HI; Bg, *Bgl*II; R, *Eco*RI. In the experiments described here, we have used primarily two recombinant plasmids as templates for *in vitro* transcription experiments. One, pHB8 (provided by T. Maniatis) contains a 7.5-kb *Hin*dIII fragment (extending from 0 to 7.5) inserted into pBR322. This fragment contains the entire β-globin gene, as well as two *Alu*I repeat sequences lying downstream from it. The other, pHB9.3, contains a 9.3-kb *Bam*HI fragment (from abou3 to 12 kb) subcloned into pBR322 from a λ phage recombinant, λHβG3 (ref. 5) (obtained from T. Maniatis).

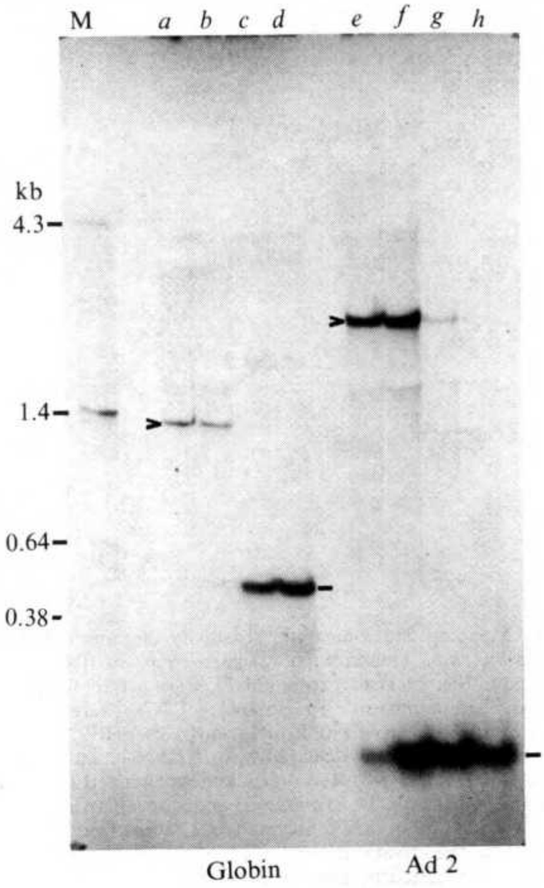

Fig. 2 Response of various promoters to alterations in the concentration of HeLa whole-cell lysate in *in vitro* reaction mixtures. HeLa whole-cell lysates were prepared as described elsewhere[13,20] except that the final dialysis buffer was 40 mM Tris-HCl *p*H 7.9, 100 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 2 mM dithiothreitol, 17% glycerol. The protein concentration of the lysate used in these experiments was ~25 mg ml^{-1}, although similar results have been obtained with less concentrated lysates. Transcription reactions (25 μl total volume) were carried out as described elsewhere[20] except that the concentration of each ribonucleotide triphosphate was 50 μM, the radioactive precursor was [α-^{32}P]GTP (5 μCi per reaction; NEN), creatine phosphate was present at 4 mM, and the DNA concentration was 90 μg ml^{-1}. The concentrations of the various buffer components were determined entirely by the amount of lysate added to each reaction mixture. The samples shown in *a*–*d* were obtained from reaction mixtures containing pHB8 cleaved with *Eco*RI as template, while the Ad2-derived recombinant pHIIIB*, cleaved with *Kpn*I, was the template in the reaction mixtures represented in *e*–*h*. The Ad2 fragment in this plasmid extends from 16.4 to 31.5 map units (1 map unit (m.u.) = 360 bp) and contains the RNA polymerase II late promoter (16.5 m.u.), as well as the two small viral-associated (VA) genes (29–30 m.u.), which are transcribed by RNA polymerase III. Cleavage with *Kpn*I (23.5 m.u.) leads to the synthesis of a 2.5-kb RNA polymerase II run-off transcript. The RNA polymerase III product, which represents primarily transcription of the VA I gene[21], is ~160 nucleotides. The amount of lysate added to each reaction mixture was: 15 μl (lanes *a* and *e*), 12 μl (lanes *b* and *f*), 9 μl (*c*, *g*), or 6 μl (*d*, *h*). After incubation at 30 °C for 60 min, RNA was extracted, denatured with glyoxal, and one-quarter of each sample resolved by electrophoresis through a 1.4% agarose gel. The autoradiograph was obtained by exposure of the dried gel to Kodak XAR-5 film for 24 h (without intensifying screens). If quantitation was required, gels were scanned with a Gilford densitometer. RNA polymerase II products are indicated by the arrowheads, RNA polymerase III products by the short lines. The marker (lane M) consists of run-off RNAs obtained from *in vitro* transcription of the plasmid p04, as described previously[22].

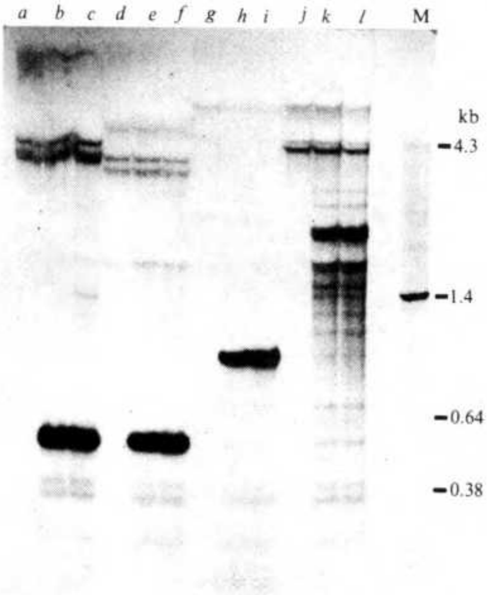

Fig. 3 Mapping and α-amanitin sensitivity of transcripts synthesized *in vitro*. Transcription reactions were carried out as described in Fig. 2 legend, except that 7.5 μl of extract were used in each reaction mixture. The following DNAs were used as templates: pHB8 cleaved with *Eco*RI (lanes *a–c*), pHB9.3 cleaved with *Eco*RI (*d–f*), pHB9.3 cleaved with *Bgl*II (*g–i*) and pHB9.3 cleaved with *Bam*HI (*j–l*). Reaction mixtures contained 200 (lanes *a*, *d*, *g* and *j*), 1 (*b*, *e*, *h* and *k*) or 0 (*c*, *f*, *i* and *l*) μg ml^{-1} α-amanitin. The 1.4-kb β-globin RNA polymerase II transcript is barely detected at this lysate concentration (lane *c*); its synthesis, however, is inhibited by a low concentration of α-amanitin, as expected (compare lanes *b* and *c*). Accumulation of the 510-nucleotide transcript is inhibited only by the higher concentration, as expected for an RNA polymerase III product (lanes *a* and *b*). If the 510-nucleotide RNA does indeed arise from transcription initiation in the β-globin-distal *Alu*I repeat, then the same transcript should be obtained when pBH9.3, which lacks sequences 5′ to the β-globin gene (see Fig. 1), is cleaved with *Eco*RI and used as template. Lanes *d–f* show that this is indeed the case. Further evidence that this RNA initiates at the deduced site was obtained by analysing RNAs transcribed from DNA cleaved with *Hin*dIII or *Bgl*II. The former results in synthesis of a 630-nucleotide run-off transcript (Fig. 4*e–g*), while the latter produces a 910-nucleotide transcript (Fig. 3*g–i*). (The high-molecular weight α-amanitin-resistant species result from end-labelling of DNA.)

Figures 3 and 4 provide further evidence that the 510-nucleotide RNA does in fact arise from transcription initiation by RNA polymerase III in the *Alu*I repeat sequence distal to the β-globin gene. Specifically, the 'run-off' transcription assay[15] was used to map the RNA start site. Transcripts sensitive to high, but not low, levels of α-amanitin and ranging in size from 510 to 910 nucleotides, were detected. These are the sizes expected if transcription begins at the 5′ end of the *Alu*I repeat sequence, and continues to the end of the DNA fragment used as templates.

RNA polymerase III, unlike RNA polymerase II, terminates transcription *in vitro* when a stop signal is encountered (for example, see Fig. 2*e–h*). All *Alu*I-promoted transcripts previously studied terminate at sites within several hundred nucleotides of the repeated element. The largest RNA transcribed from an *Alu*-type repeat previously described is 800 nucleotides[11], and all of the approximately 10 other *Alu*I-promoted transcripts from the human α- or β-like globin clusters are <600 nucleotides long[8,9]. It was therefore rather unexpected that transcription actually continued to the end of the *Bgl*II-cleaved DNA (910 nucleotides). To determine the length of the transcribed region, pHB9.3 digested with *Bam*HI was used as template. Surprisingly, the major product of transcription was 2.3 kb, although several minor transcripts (up to 3.2 kb) were detected also (Fig. 3*j–l*). The transcripts produced from *Bam*HI-digested DNA arise from specific initiation and termination, as the same RNAs are obtained when closed circular DNA is used as template (Fig. 4*a–d*).

The function(s) that *Alu*I repetitive sequences serve in cells is unclear, although several possibilities have been discussed (for example, see refs 3 and 9). It is almost certain that at least some of the repeats that are transcribed by RNA polymerase III *in vitro* are also transcriptionally active *in vivo*. For example, specific low-molecular weight transcripts have been shown to be complementary to cloned *Alu*-type repeated DNA sequences isolated from CHO cells[11]. Also, the observation that the start point of transcription seems to correspond to the first nucleotide of the repeat sequence strongly suggests that the presence of an RNA polymerase III promoter is not coincidental[16,17]. However, it is unknown if any of the *Alu*I promoters in the β-globin locus are ever used *in vivo*.

The results presented here establish several interesting points with respect to RNA polymerase III and the possible role of *Alu*I repeats. First, RNA polymerase III has long been thought to be the enzyme responsible for synthesis of small RNAs. While most of the products of this polymerase are undoubtedly small (for example, tRNAs, 5S RNA), the results shown above

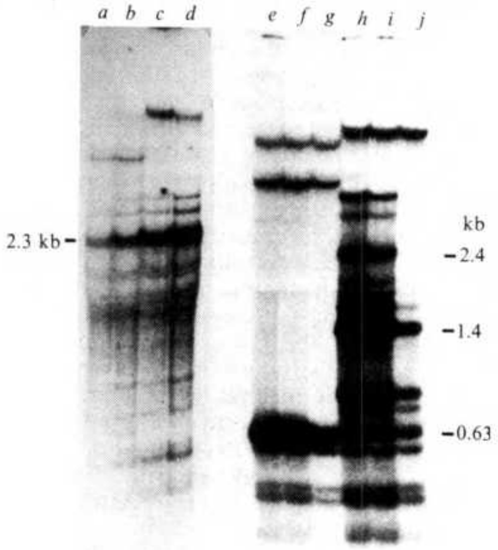

Fig. 4 Comparison of the transcription products obtained from circular and linear DNA. Transcription reactions were as described in Fig. 2 legend except that 7.5 μl of extract were used. The DNA templates were as follows: pHB9.3 cleaved with *Bam*HI, 60 μg ml^{-1} (lane *a*) or 100 μg ml^{-1} (lane *b*); pHB9.3, Form I, 60 μg ml^{-1} (*c*) or 100 μg ml^{-1} (*d*); pHB8 cleaved with *Hin*dIII, 100 μg ml^{-1} (*e–g*); and pHB8, Form I, 100 μg ml^{-1} (*h–j*). α-Amanitin (1 μg ml^{-1}) was included in two reaction mixtures (*f* and *i*) and 20 μg ml^{-1} α-amanitin was included in two others (lanes *g* and *j*). Note that the expected 630-nucleotide run-off transcript is observed with *Hin*dIII-cleaved DNA (lanes *e–g*), but the circular DNA gives rise to predominantly larger transcripts (lanes *h–j*), which arise from termination sites in pBR322. (A stretch of five consecutive T residues is probably responsible for the 2.4-kb RNA, while the 1.4-kb transcript probably results from termination at a stretch of four Ts[23].) These are the only T stretches longer than three in the region of pBR322 that is transcribed, which corresponds, for the most part, to the *tet* gene[24]. Synthesis of these transcripts is resistant to 1 μg ml^{-1} α-amantinin (for example, lanes *h* and *i*). However, an interesting response to 20 μg ml^{-1} α-amanitin (lane *j*) was observed: transcription of the smaller RNAs was inhibited only slightly, while synthesis of the larger species was suppressed almost completely. This could reflect a 'target-size' phenomenon in which polymerases that transcribe a greater DNA length have a higher probability of interacting with the drug. Alternatively, α-amanitin may exert its inhibitory effect early during transcription, and those polymerases that continue are more likely to terminate when a potential termination signal is reached, as has been suggested in the case of transcription of 5S genes[23].

establish that this enzyme can synthesize large RNAs, and that templates for such transcription exist in human cells. Second, the possibility that *Alu*I repetitive sequences might function by promoting transcription of adjacent genes is strengthened by the observation that a long stretch of DNA can be transcribed. For example, although no precedent exists for transcripts synthesized by RNA polymerase III functioning as mRNA, an RNA 2.3 kb long could encode a protein as large as molecular weight 80,000. Indeed, the presence of *Alu*I repeat sequences in cDNA transcripts of polysomal poly(A)$^+$ RNA has been reported[4]. Third, since it has been shown that the *Alu*I repetitive sequence studied here is immediately adjacent to a long (~6 kb) middle repetitive DNA sequence (~5,000 copies per genome)[18,19], we have established the novel situation in which a short, highly repetitive DNA sequence provides a promoter which results in the *in vitro* transcription of a substantial portion of a long, middle repetitive sequence. It will be of interest to determine the function of this transcription, and any role it may have in influencing the expression of the neighbouring β-globin-like genes.

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Sequence and structural homologies among type I and type II interferons

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Interferons are proteins with antiviral, antitumour and immunomodulator activities, which are secreted in response to various inducers[1]. The interferons have been classified into two categories on the basis of their biological and physical properties. Type I interferons include fibroblast interferon (IFN-β)[2,3] and the leukocyte family of interferons which is composed of at least 10 subspecies[4–8]. Each member of the type I interferons contains ~165 amino acids, is acid-stable, and competes for the same receptors[9]; furthermore, identical amino acids occupy invariant positions in 23% of their amino acid sequences[7]. In contrast, type II interferon (IFN-γ) is produced in response to mitogens and antigenic stimuli[10], contains ~146 amino acid residues[11], is not acid-stable, and displays no measurable binding to type I interferon receptors[9]. The nucleotide sequence of the cDNA coding for IFN-γ has recently been reported[11], and no statistically significant sequence homology was detected between the deduced amino acid sequences of IFN-γ and any of the type I interferons[11,12]. To determine whether there might be structural similarities between IFN-γ and the type I interferons, we have conducted a predictive analysis of the secondary structure of these proteins[13,14]. IFN-γ as well as each of the type I interferons contain a segment with a high potential to form an amphiphilic α-helix of approximately the same length and hydrophobic/hydrophilic balance (Fig. 1). As we report here, the identification of these segments generates an alignment of sequences which reveals previously undetected sequence homologies among the type I and II interferons (Fig. 2). Thus, we propose that there is a common evolutionary ancestor for both type I and type II interferons.

Phylogenetic relationships among proteins are often elucidated by demonstrating that the protein sequences may be aligned so that a larger number of identical amino acids occupy respective positions along the polypeptide chain (homologies) than might be expected from a chance alignment of unrelated proteins. However, when the relationships are distant, deletions (gaps) or additions of residues may occur between homologous regions in the amino acid sequences of the proteins. This hampers the proper alignment of two marginally related sequences because the introduction of gaps increases the number of homologies which would be expected by chance alignment of sequences[15], and also increases by orders of magnitude the number of possible alignments which must be searched for homologies. Clearly, a nonrandom method which would direct the proper alignment of portions of the sequences of related proteins would be extremely useful. When the three-dimensional structures of two proteins have been solved by

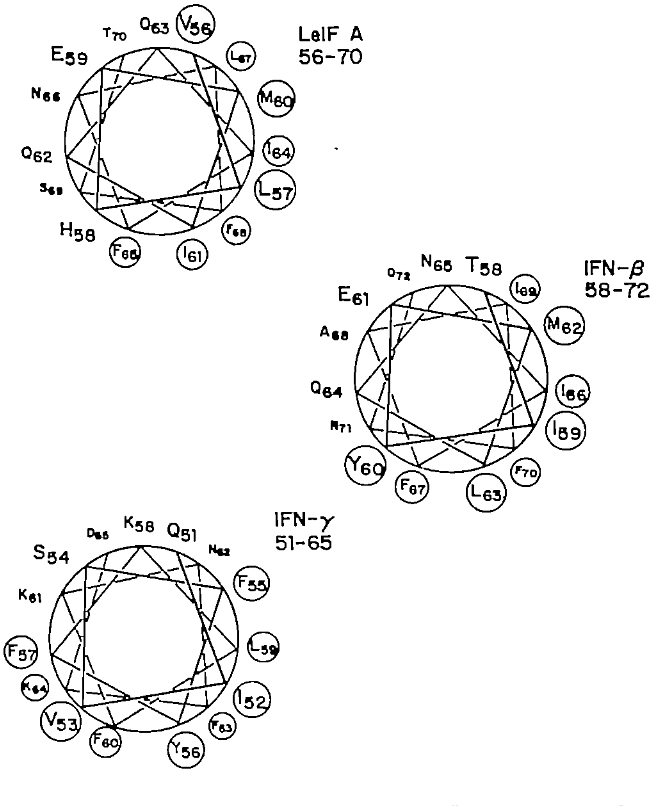

Fig. 1 Axial helical projection[19] of LeIF A[7] (56–70), IFN-β[3] (58–72) and IFN-γ[11] (51–65).

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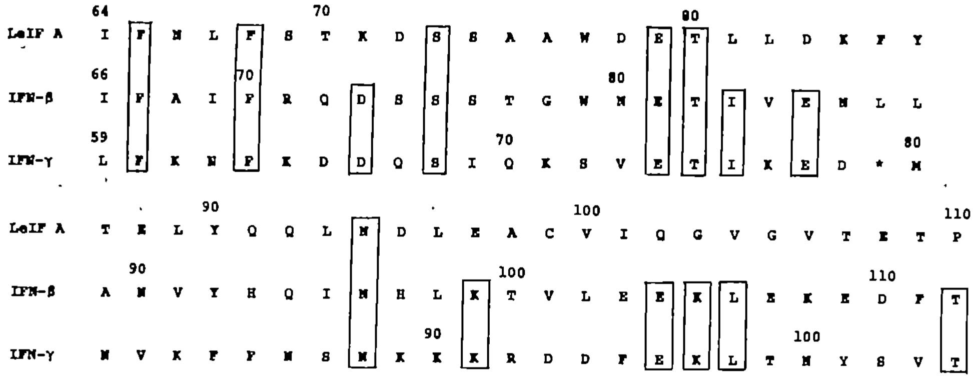
64 70 90
LeIF A I F N L F S T K D S S A A W D E T L L D K F Y
66 70 80
IFN-β I F A I F R Q D S S S T G W N E T I V E N L L
59 70 80
IFN-γ L F K N F K D D Q S I Q K S V E T I K E D * M
90 100 110
LeIF A T E L Y Q Q L N D L E A C V I Q G V G V T E T P
90 100 110
IFN-β A N V Y H Q I N H L K T V L E E K L E K E D F T
90 100
IFN-γ N V K F F N S N K K K R D D F E K L T N Y S V T

Fig. 2 Amino acid sequences of LeIF A[7] (63–104), IFN-β[3] (65–106) and IFN-γ[11] (59–106). Homologous residues are boxed.

X-ray crystallography, relatedness can be demonstrated by aligning the primary structures based on secondary and tertiary structural similarities between the proteins[16,17]. Unfortunately, the three-dimensional structures of most proteins are not known so we are attempting to make alignments based on predicted secondary-structural similarities. However, most existing methods for secondary structure prediction do not allow precise enough predictions of the positions of initiation or termination of a given secondary structural element[18] to allow unambiguous alignments. Some of these ambiguities might be eliminated by considering the distribution of hydrophobic and hydrophilic residues within a predicted secondary structure. In particular, amphiphilic α-helices have hydrophobic and hydrophilic residues on opposite faces of the α-helix and are known to occur on the surfaces of globular proteins[19] and lipoproteins[20,21]. Furthermore, the occurrence of amphiphilic α-helices may be predicted by locating the portions of amino acid sequences whose hydrophobic and hydrophilic residues repeat in a pattern such that they are segregated on opposite sides of a helix when the chain adopts a helical conformation[19,22]. Thus two or more protein sequences might be aligned at sub-sequences which share a hypertensional to form amphiphilic α-helices of similar length and hydrophobic/hydrophilic balance.

LeIF A, IFN-β and IFN-γ have several segments which are predicted to form amphiphilic α-helices (LeIF A was chosen as representative (Fig. 3) of the leukocyte interferons). Of the amphiphilic α-helical segments in these proteins, one in particular is of uniform length and uniform hydrophobic/hydrophilic balance among all three interferons. These segments, which span residues 56–70 in LeIF A, 58–72 in IFN-β and 51–65 in IFN-γ (Fig. 1), also have high potentials to form α-helices as determined by the Chou and Fasman[14] parameters ($P\alpha = 1.09$, 1.10 and 1.04, respectively). When the sequences of the interferons are aligned so that these regions with high amphiphilic α-helical potential coincide, sequence homologies are observed between residues 64–110 (LeIF A), 66–112 (β) and 59–104 (γ). If a space is inserted between positions 79 and 80 of IFN-γ, IFN-γ and IFN-β share 14 invariant residues between residues 59 and 104 (γ numbering), and an additional 4 residues are structurally conserved (Leu for Ile; Lys for Arg; Asp for Asn; Tyr for Phe). Thus, there is 30% sequence homology between β and γ interferon (Fig. 2) in this region and 39% homology if conservative replacements are included; the corresponding values for the homologies between IFN-γ and LeIF A are 13% and 24%, respectively. Similar degrees of homology are observed between IFN-γ and the other leukocyte interferons (Fig. 3).

The significance of our findings was tested by examining the degree of homology to be expected between randomized sequences of IFN-γ and the type I interferons[23]. A program was written which performed the following operations: (1) Two random sequences were generated with the frequency of occurrence of amino acids identical to that of the proteins under consideration. (2) A segment of desired length m was selected from the first random sequence. (3) This segment was overlayed on the first m residues of the second (longer) sequence of length n and the number of matches determined. (4) A single gap was inserted into the pair of sequences and the number of homologies noted. (5) Step 4 was repeated for all $2(m-1)$ possible locations of the gap. (6) The segment of length m was moved one residue to the right and steps (3), (4) and (5) repeated. (7) Step 6 was repeated until the end of the longer sequence was reached.

The maximum number of homologies observed for the $2(m-1)(n-m+1)$ possible alignments generated by the above process was noted. Steps (1)–(7) were repeated 1,000 times. The mean and standard deviation of the maximum number of homologies observed were computed. The quantities provide a measure of the degree of homology to be expected for the best alignment (that alignment with the highest number of homologies among all possible alignments) of a segment of m residues on another segment of n residues (with one gap) when the amino acids are randomly distributed. Using this program, we find that the mean and standard deviation for the number of

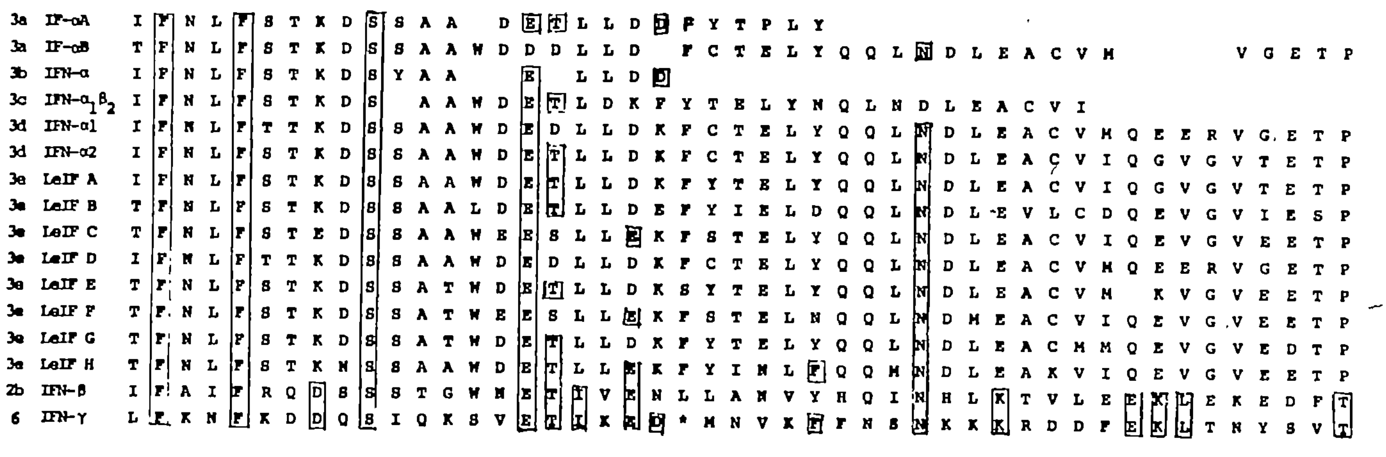
Ref. 64 70 80 90 100 110
3a IF-αA I F N L F S T K D S S A A D E T L L D D F Y T P L Y
3a IF-αB T F N L F S T K D S S A A W D D D L L D F C T E L Y Q Q L N D L E A C V H V G E T P
3b IFN-α I F N L F S T K D S Y A A E L L D D
3c IFN-α1β2 I F N L F S T K D S A A W D E T L D K F Y T E L Y N Q L N D L E A C V I
3d IFN-α1 I F N L F T T K D S S A A W D E D L L D K F C T E L Y Q Q L N D L E A C V H Q E E R V G E T P
3d IFN-α2 I F N L F S T K D S S A A W D E T L L D K F C T E L Y Q Q L N D L E A C V I Q G V G V T E T P
3e LeIF A I F N L F S T K D S S A A W D E T L L D K F Y T E L Y Q Q L N D L E A C V I Q G V G V T E T P
3e LeIF B T F N L F S T K D S S A A L D E T L L D E F Y I E L D Q Q L N D L E V L C D Q E V G V I E S P
3e LeIF C T F N L F S T E D S S A A W E E S L L E K F S T E L Y Q Q L N D L E A C V I Q E V G V E E T P
3e LeIF D I F N L F T T K D S S A A W D E D L L D K F C T E L Y Q Q L N D L E A C V H Q E E R V G E T P
3e LeIF E T F N L F S T K D S S A T W D E T L L D K S Y T E L Y Q Q L N D L E A C V H K V G V E E T P
3e LeIF F T F N L F S T K D S S A T W E E S L L E K F S T E L N Q Q L N D H E A C V I Q E V G V E E T P
3e LeIF G T F N L F S T K D S S A T W D E T L L D K F Y T E L Y Q Q L N D L E A C H M Q E V G V E D T P
3e LeIF H T F N L F S T K N S S A A W D E T L L E K F Y I N L F Q Q H N D L E A K V I Q E V G V E E T P
2b IFN-β I F A I F R Q D S S S T G W N E T I V E N L L A N V Y H Q I N H L K T V L E E K L E K E D F T
6 IFN-γ L F K N F K D D Q S I Q K S V E T I K E D * M N V K F F N S N K K K R D D F E K L T N Y S V T

Fig. 3 Partial amino acid sequences of all human interferons thus far reported in the literature. Homologous residues are boxed.

homologies between randomized sequences of IFN-β and a 46-residue segment of IFN-γ is 9.0 ± 1. Further, the frequency of finding 14 or more homologies is less than 10^{-4}. Notably, although 6 homologies between LeIF A and IFN-γ (Fig. 2) are not statistically significant, these homologous residues are also concerved in IFN-β. The frequency of finding 6 homologies within the 14 homologous positions contained in randomized 46-residue sequences with the amino acid composition of LeIF A and IFN-γ is 4×10^{-3}. (A gap was not included in this calculation because the position of the gap was determined by the prior positioning of the β and γ sequences. If a gap is included the probability is 1.32×10^{-2}.) We conclude that the type I and type II interferons are indeed related and probably arose from a common ancestor.

In contrast to our proposed sequence alignment between IFN-γ and LeIF A, Epstein[12] observed only 14 sequence homologies spread throughout the entire sequences of these two proteins. This is below the value expected for the best random alignment of the entire sequences and insertion of a single gap (16.9 ± 1.7), suggesting that the statistical relatedness of this alignment is far less than the alignment suggested by us.

Our results clearly show the potential for using predicted structural features to align distantly related sequences. This method has been used to demonstrate that a feature common to all interferons thus far sequenced is a segment of 15 residues with a high potential to form an amphiphilic α-helix followed by a region of high sequence homology. The specific amino acid sequence of a large portion of these segments with high amphiphilic potential appears to be of little consequence as long as its amphiphilic character is conserved, suggesting that this region might serve a structural role in stabilizing the surface of these proteins. Zav'yalov and Denesyuk[24] and Sternberg and Cohen[25] have also suggested that this region is helical and might serve a structural role in the α- and β-interferons.

The degrees of homology between residues 59 and 104 (IFN-γ numbering) among IFN-γ, IFN-β and LeIF A suggest that the IFN-γ gene separated from the IFN-β gene some time after the separation between leukocyte and fibroblast interferon genes. The lower degree of homology between IFN-γ and LeIF A than between IFN-β and LeIF A clearly shows that the mutation rate for IFN-γ has been greater than that for the leukocyte interferons and IFN-β, consistent with type I interferon having separate receptors from the IFN-γ receptor[9]. The observation that the highest concentration of homologies is in a 46-residue portion of IFN-γ and IFN-β suggests that IFN-γ might be a product of the insertion of a short segment of DNA coding for IFN-β into a gene coding for an unrelated protein. IFN-γ gene contains one or more introns[11], providing a possible mechanism for this insertion.

We thank E. Knight for helpful discussions.

Since submission of this paper the complete sequence of the human immune interferon (IFN-γ) gene including the exon and intron structure has been published[26]. A single exon of 61 amino acids in length contains the entire segment predicted by us here to form an amphiphilic α-helix as well as 44 of 46 amino acids we also show to be homologous. Furthermore, only 9 of the 61 amino acids in this exon do not appear in the structurally and functionally homologous unit proposed by us. This observation further supports previous suggestions[27,28] that exons code for functionally competent domains of proteins.

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Nucleotide sequence of $\gamma\delta$ resolvase gene and demonstration that its gene product acts as a repressor of transcription

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The related transposons $\gamma\delta$ and Tn*3* each encode an efficient site-specific recombination system essential for transposition[1,2]. The recombination occurs at a particular site, *res*, located within the intercistronic region between the two divergently transcribed genes that are required for transposition[2]. The product of one of these genes, resolvase, catalyses the recombination at *res*. Recently, *in vitro* site-specific recombination has been demonstrated using purified resolvase and a model substrate containing two *res* sites[3]. Genetic analysis of mutants in the $\gamma\delta$/Tn*3* *tnpR* gene, which encodes the resolvase protein, suggested that resolvase regulates the frequency of transposition in addition to catalysing recombination[4–6]. Analysis of element-encoded proteins in minicells indicated a direct effect of resolvase on the levels of the element-encoded transposase, as well as on its own expression[7]. Here we report the sequence of the gene encoding $\gamma\delta$ resolvase and compare the predicted amino acid sequence with that of the functionally interchangeable Tn*3* protein[6]. In addition, we have determined the sites at which transcription of the transposase and resolvase genes initiates *in vitro* and have demonstrated that purified resolvase protein specifically represses transcription of both transposition related genes in this *in vitro* transcription system.

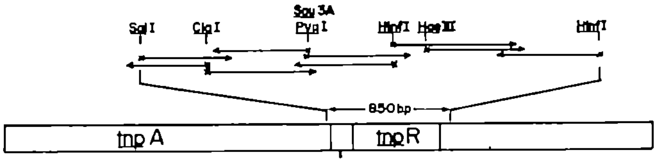

Fig. 1 Genetic map of $\gamma\delta$ and the region sequenced. The element is drawn with the γ end[17] towards the right. Transcription of *tnpA* and *tnpR* is divergent from the central intercistronic region. The right arm appears to encode a 79,000 molecular weight polypeptide of unknown function (unpublished observation). The locations and directions of sequence determinations are indicated by arrows with the 5′ labelled end indicated by X. The DNA was labelled and sequenced as described by Maxam and Gilbert[18]. The $\gamma\delta$ *tnpR* sequences were determined using fragments from the plasmid pOX14, a $\gamma\delta$ transposition into pBR322[17].

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```
                                     ILE                                                                 ILE
Tn3  C      A G                  A             T   G       C                   G                     A C
γδ  TTGATTTAGGATACATTTTT ATG CGA CTT TTT GGT TAC GCA CGG GTA TCA ACC AGC CAG CAA TCT CTC GAT ATT CAG GTT  80
                         MET ARG LEU PHE GLY TYR ALA ARG VAL SER THR SER GLN GLN SER LEU ASP ILE GLN VAL

                                                                                             THR
Tn3  A A   G           T       G   A       T   C               C               C              CA
γδ   CGG GCA CTC AAA GAC GCA GGC GTG AAA GCA AAT CGC ATC TTT ACT GAC AAG GCA TCG GGC AGT TCA AGC GAT CGG 156
     ARG ALA GLU LYS ASP ALA GLY VAL LYS ALA ASN ARG ILE PHE THR ASP LYS ALA SER GLY SER SER SER ASP ARG

     GLU
Tn3  G             T                                       T       T C             G   C       T C     C
γδ   AAA GGG CTG GAC TTG CTG AGG ATG AAG GTG GAG GAA GGT GAC GTC ATC TTG GTG AAG AAA CTT GAC CGC CTT GGG 232
     LYS GLY LEU ASP LEU LEU ARG MET LYS VAL GLU GLU GLY ASP VAL ILE LEU VAL LYS LYS LEU ASP ARG LEU GLY

                                                                             ALA VAL
Tn3        C   C   C               A C G                   T   T   G         G G G                 C
γδ   CGC GAT ACT GCT GAC ATG ATC CAG TTA ATA AAA GAG TTT GAC GCC CAA GGT GTA TCC ATT CGG TTT ATT GAT GAC 308
     ARG ASP THR ALA ASP MET ILE GLN LEU ILE LYS GLU PHE ASP ALA GLN GLY VAL SER ILE ARG PHE ILE ASP ASP

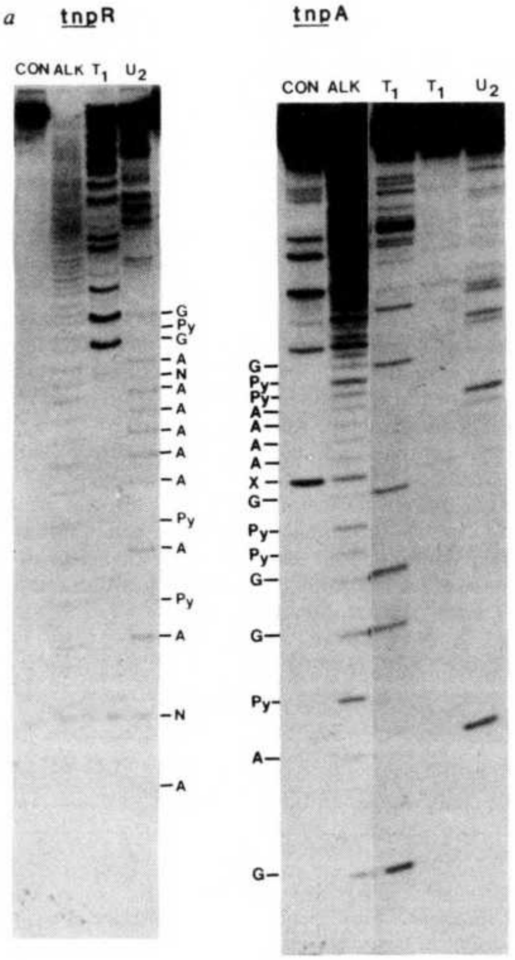
                             ASP         GLN                                                         ARG
Tn3    G               C   T   T       G C         G       C   C   G   G   T       A       T       C  G
γδ   GGA ATC AGT ACC GAT GGG GAG ATG GCT AAA ATG GTT GTC ACT ATT CTA TCT GCA GTG GCC CAG GCA GAA CGA CAG 384
     GLY ILE SER THR ASP GLY GLU MET GLY LYS MET VAL VAL THR ILE LEU SER ALA VAL ALA GLN ALA GLU ARG GLN

                                                         LYS LEU         ILE LYS             ARG     THR
Tn3    G   C           C   G       G   C   A   G   A      A  CTG         A C AAA       C C C  GG C T  CC
γδ   AGA ATA CTA GAG CGT ACC AAT GAA GGT CGC CAA GAG GCA ATG GCA AAA GGA GTT GTT TTT GGT AGA AAA AGA AAA 460
     ARG ILE LEU GLU ARG THR ASN GLU GLY ARG GLN GLU ALA MET ALA LYS GLY VAL VAL PHE GLY ARG LYS ARG LYS

     VAL         ASN VAL         THR LEU HIS     LYS     THR         THR GLU     ALA HIS GLN LEU SER
Tn3  G G   C   G A    T    G C G  CG C T CAT   G A     C ACT       A A G G A   T G T C T C G C C  G
γδ   ATA GAT AGA GAT GCA GTA TTA AAT ATG TGG CAA CAG GGG TTA GGT GCC TCA CAT ATA TCA AAA ACA ATG AAT ATT 536
     ILE ASP ARG ASP ALA VAL LEU ASN MET TRP GLN GLN GLY LEU GLY ALA SER HIS ILE SER LYS THR MET ASN ILE

                                 ILE LEU GLU ASP GLU ARG ALA SER***
Tn3    C   C   C   G   T         A T C T G T   C GAA  GG CCC TCGTC TACGCCTA T TT TAG TTAA GT A G T A AAT
γδ   GCT CGT TCA ACA GTA TAT AAA GTA ATA AAT GAA AGC AAC TAA CATAAAACGTTGAGTGTGGAATTGAGTGTTACTTCAAAGTCTA 621
     ALA ARG SER THR VAL TYR LYS VAL ILE ASN GLU SER ASN ***
```

Fig. 2 Sequence of $\gamma\delta$ resolvase. The DNA sequence of the *tnpR* gene is shown along with the predicted amino acid sequence. The nucleotides and amino acids at which the Tn*3* *tnpR* gene sequence[8] differs from the $\gamma\delta$ sequence are indicated.



b

```
                                                     -35                  ↓ -10      +1
                                                     ••• •                •• • ••    ∿∿∿→
GTC GAC AGG CAT ATCAGGCTCCCTACCTGACAGTTATTCATTAACAATTTTGCAACCGTCCGAAATATTATAAATTATCGCACACATAAAAACAGTGCTG
CAG CTG TCC GTA ATGTCCGAGGGATGGACTGTCAATAAGTAATTGTTAAAACGTTGGCAGGCTTTATAATATTTAATAGCGTGTGTATTTTTGTCACGAC
ASP VAL PRO MET         '                   '            ←∿∿∿        •••••••                    ••
                       +40                 +20               +1         ↑                     -35
                                                                          -10
          tnpA

+20                +40                 +60                 +80               tnpR
'                   '                   '                   '               MET ARG LEU PHE GLY TYR ALA
TTAATGTCTCTATTAAATCGATTTTTTGTTATAACAGACACTGCTTGTCCGATATTTGATTTAGGATACATTTTT ATG CGA CTT TTT GGT TAC GCA
AATTACACAGATAATTTAGCTAAAAAACAATATTGTCTGTGACGAACAGGCTATAAACTAAATCCTATGTAAAAA TAC GCT GAA AAA CCA ATG CGT
```

Fig. 3 *a*, Sequence of the major *tnpR* and *tnpA* transcripts. *In vitro* transcription reactions (20 μl) consisted of 0.1 pmol of the *Sal*I/*Pvu*I fragment containing the *tnpR*/*tnpA* intercistronic region, 10 mM Tris–HCl *p*H 7.5, 150 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 100 μM unlabelled NTPs, 0.9 μg *E. coli* RNA polymerase and in separate reactions 10 μM [γ-^{32}P]ATP, or [γ-^{32}P]GTP (specific activity 1,000 Ci $mmol^{-1}$, NEN). The mixtures were incubated at 37 °C for 20 min, and then stopped by addition of SDS to 0.1% before phenol extraction. After addition of an equal volume of 80% formamide in TBE, the samples were heated to 95 °C for 2 min, and applied to a 1.5-mm thick, 10% acrylamide/7 M urea TBE gel[18]. After electrophoresis and autoradiography, bands corresponding to the major transcription products were excised and eluted. These 5′-labelled transcripts were subjected to partial enzymatic degradation and fractionation on 25% acrylamide/7 M urea gels as described by Donis-Keller[11]. CON, undigested transcript; ALK, partial alkaline hydrolysis; T_1, ribonuclease T_1, which cleaves specifically after G residues; U_2, ribonuclease U_2, which cleaves specifically after A residues in the conditions used here. Note that the preparative gel used to isolate transcription products was unable to resolve multiple *tnpR* species. However, analysis of individual bands within the *tnpA* cluster (see Fig. 4*a*) indicated a common 5′ terminal sequence. *b*, The nucleotides at which *tnpA* and *tnpR* transcription initiates are indicated (wavy arrow). The bars indicate the −10 and −35 regions proposed to interact with RNA polymerase and the dots indicate homology between those nucleotides and the canonical *E. coli* promoter sequence of Rosenberg and Court[13]. The positions at which the *res* site is cleaved *in vitro* by resolvase are indicated by the arrows[2].

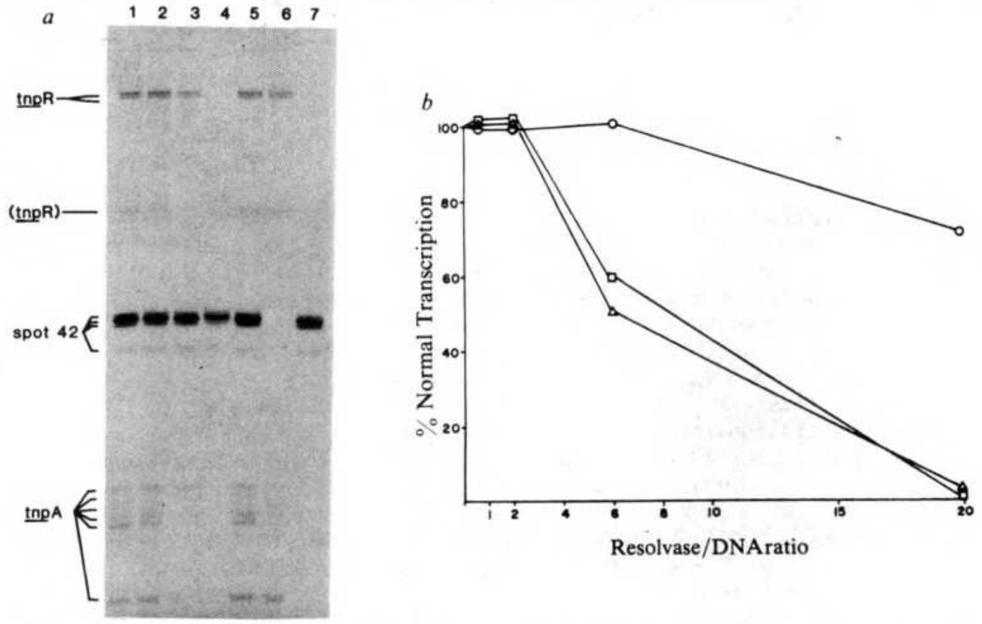


Fig. 4 *a*, Specific repression by $\gamma\delta$ resolvase. Transcription was performed as in Fig. 3 except that [α-^{32}P]GTP (specific activity 50 Ci mmol^{-1}; NEN) was used. As an internal control, the efficiency of transcription 0.1 pM of a 165 bp fragment containing the spot 42 promoter and terminator (C. N. Joyce, personal communication) was also monitored in each reaction. Lanes 1–5 contain both templates, lane 6 only the *res* template and lane 7 only the spot 42 template. Resolvase was added and the reactions incubated for 5 min before the addition of RNA polymerase. Lane 1, 0.1 pmol resolvase; lane 2, 0.3 pmol resolvase; lane 3, 0.6 pmol resolvase; lane 4, 2.0 pmol resolvase; and lanes 5–7, no added resolvase. The identity of each indicated transcript was confirmed by two-dimensional T_1-ribonuclease fingerprinting and by secondary analysis[19]. The multiple bands result from heterogeneity at the 3′ ends of the run-off transcripts. The band labelled (*tnpR*) is a prematurely terminated *tnpR* transcript. *b*, Densitometric analysis of transcriptional repression by resolvase. Lanes 1–5 were scanned and the intensities of each peak determined relative to that in the absence of resolvase (lane 5). The results were then plotted versus the molar ratio of resolvase monomers to *res* template. Spot 42 transcription is indicated by ○, *tnpR* by □ and *tnpA* by △. Decreases in intensity were identical for each band within the *tnpA* cluster.

Figure 1 shows a map of $\gamma\delta$ and of the region sequenced. The DNA sequence has revealed a single open reading frame which is capable of encoding a slightly basic 183-residue polypeptide, corresponding to the $\gamma\delta$ resolvase protein (Fig. 2). The amino acid sequence of $\gamma\delta$ resolvase shows 80% homology with that of the analogous Tn*3* protein[8], the homology being particularly extensive for the N-terminal two-thirds of the sequence. In view of the interchangeability of the $\gamma\delta$ and Tn*3* proteins in both recombination and repressor roles[2], the high degree of homology in the N-terminal region may indicate a conservation of the DNA binding site. Interestingly, the protein which catalyses recombination in the *hin* site-specific recombination system of *Salmonella typhimurium* (which is involved in the expression of flagellar proteins)[9] is homologous to $\gamma\delta$ resolvase, and exhibits a similar pattern of sequence conservation. Resolvase and the *hin* protein are not interchangeable for recombination functions (unpublished observation).

Considerable genetic evidence has suggested that $\gamma\delta$/Tn*3* resolvase regulates expression of element-encoded proteins (resolvase and transposase) at the transcriptional level[10]. We have determined the sites at which transcription of these two genes initiates *in vitro*. A purified 303 bp *Sal*I/*Pvu*I fragment containing all of the intercistronic region between *tnpA* and *tnpR* was used in transcription experiments with *Escherichia coli* RNA polymerase and γ-^{32}P labelled purine triphosphates. The resulting transcripts were sequenced by the method of Donis-Keller[11] (see Fig. 3*a*). Determination of the two start sites allows identification of the regions of the DNA sequence suggested to be involved in RNA polymerase interactions[12] (Fig. 3*b*); homologies between these regions and the canonical *E. coli* promoter sequence[13] are indicated by dots. The -10 region of the two promoters overlap one another, and contain the site at which resolvase cleaves the *res* site to initiate strand exchange[14] (indicated by the arrows in Fig. 3*b*). This cleavage is observed at a significant frequency only in special conditions and thus is unlikely to have a role in regulation.

The divergent transcription of regulatory proteins and of the genes they control has been described previously. However, in the *araC*/*araBAD* operons of *E. coli*[15] as well as in the rightward region of bacteriophage λ (ref. 16) the nucleotides at which divergent transcription initiates are separated by more than 50 base pairs (bp). In the *res* system, RNA polymerase competition for binding at the two promoters may represent an additional regulatory complexity.

Genetic evidence has suggested a role for resolvase in regulation of the *tnpR* and *tnpA* genes. The data in Fig. 4 demonstrate that transcription from the *Sal*I/*Pvu*I fragment used in the previous experiment can be specifically repressed by the addition of resolvase. As an internal control, we monitored the efficiency of transcription of a promoter-containing fragment not expected to be regulated by resolvase (the spot 42 transcript). The resolvase concentration at which a significant reduction of transcription of the *Sal*I/*Pvu*I fragment occurred (~6 protein monomers per template) corresponds closely to that required for resolution (~6–8 monomers per resolution site)[3]. *In vitro* recombination studies have identified the two sites at which resolvase mediates strand cleavage to initiate recombination[14]. Their location within the Pribnow boxes for *tnpA* and *tnpR* transcription strongly suggests that resolvase regulates expression of transposition functions and resolves intermediates in the transposition pathway by acting at a single site or group of sites located within the intercistronic region between the divergently transcribed transposase and resolvase genes.

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MATTERS ARISING

Porphyropsin in retina of four-eyed fish, *Anableps anableps*

A RECENT investigation[1] reports the same visual pigments in the dorsal and ventral regions of *Anableps* retina. I present here some previously unpublished studies that demonstrate the existence of porphyropsin as well as rhodopsin in this species. *Anableps* is therefore similar to two related poeciliids, *Belonesox belizanus* and *Mollienesia latipinna*[2]. The *Anableps* used in the present work were examined only 3 days after collection from their native waters in Guiana, whereas the fish used by Avery and Bowmaker[1] originated from a fish supplier in London. Mixed rhodopsin–porphyropsin retinas are highly variable in composition; this variability probably affects the cone system also[3] and often depends on environmental factors[4]. For example, it has been shown that *Belonesox* may lose much of its porphyropsin if kept in unnaturally bright surroundings[5] and that its rhodopsin–porphyropsin balance shifts markedly with season and local habitat[6]. Other species are known to behave similarly[4]. Therefore, Avery and Bowmaker's results do not exclude the possibility that in its natural habitat *Anableps* may have appreciable proportions of porphyropsin that could be segregated in its dorsal retina.

Specimens of *Anableps* were flown directly from Guiana to Miami. A total of 3 days elapsed between the time of netting and delivery (August 1963) to my laboratory in the Bascom Palmer Eye Institute, University of Miami, Florida. After overnight dark-adaptation at 80°F, 11 fish ranging in standard length from 7 to 10 cm were killed by decapitation. The retinas were then dissected out and the photoreceptor outer segments prepared by sucrose flotation[2]. Further procedures used have been described previously[2]. The visual pigment was extracted in three successive 0.5-ml volumes of 2% digitonin (Merck), and each extract was buffered to *p*H 8.8 with sodium borate.

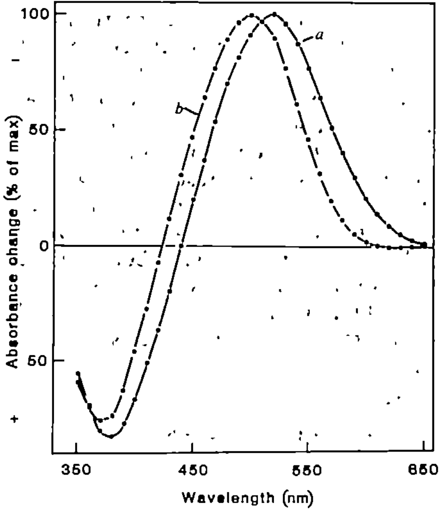

Fig. 1 Analysis of *Anableps* visual pigments. Curve *a* represents the difference spectrum obtained by bleaching a digitonin extract with light of wavelength 660 nm in the equipment described by Bridges[2]: the maximum absorbance loss was 0.077 at 519 nm. After several subsequent exposures to red light (660 and 620 nm), the visual pigment was completely bleached by exposure to yellow light (>440 nm). Curve *b* represents the difference spectrum for this final irradiation: the maximum absorbance loss was 0.0944 at 500 nm (see text for details).

The data presented here are for the first extract from the above procedure, which had a peak absorbance of 0.57 at 507 nm. The extract had more than one visual pigment—this was demonstrated by exposing it to a series of irradiations at wavelengths ranging from 660 to 620 nm, then finally to non-isomerizing yellow light of wavelengths >440 nm. Figure 1 curve (*a*) shows the difference spectrum for an initial exposure to 660 nm light. The amount bleached represents 14% of the total visual pigment, and the difference spectrum has a λ_{max} at 519 nm, close to that of porphyropsin. The difference spectrum for the final exposure to yellow light (representing 16% of the total pigment) had a λ_{max} at 500 nm, typical of rhodopsin. As Fig. 1 shows, the λ_{max} of the bleaching product also shifted to shorter wavelengths. A single fish from this group gave similar results: the digitonin extract had a peak absorbance of 0.12 at 506 nm. As the cone pigments of fishes do not survive digitonin extraction, it seems that the *Anableps* used here contained a mixture of rhodopsin and porphyropsin in their retinas.

The λ_{max} of *Anableps* rhodopsin was calculated[4] to be identical to that reported for *Belonesox*[2] that is, 498 nm, and the proportion of porphyropsin was 25–30%. The presence of porphyropsin may have accounted for the elevated λ_{max} of 506 nm previously assigned to the rhodopsin of this species[7]. The apparent agreement with Avery and Bowmaker's mean rod λ_{max} may be fortuitous, because *in situ* values are often displaced to longer wavelengths[8,9].

Thus, *Anableps* appears to be similar to several of its close relatives in having a mixture of rhodopsin and porphyropsin. Extensive studies of other species have established that such mixtures may vary widely in composition, and that ambient illumination is an important controlling factor[4]. Therefore, further studies of *Anableps* in its natural habitat or in a variety of controlled lighting conditions are needed before a final conclusion on its dorso-ventral distribution of visual pigments can be reached.

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AVERY AND BOWMAKER REPLY—Bridges presents data from partial bleaching experiments to show that the rod pigment of *Anableps* (λ_{max} = 506–507 nm) is actually a mixture of rhodopsin (λ_{max} = 498 nm) and a porphyropsin (λ_{max} = 519 nm), and is not a pure rhodopsin as reported by Schwanzara[1]. In our recent investigation of the dorso-ventral distribution of visual pigments in *Anableps*[2], we suggested from the shape of the absorbance spectra of the rods, obtained by microspectrophotometry, that the pigments were probably rhodopsins, but we are not surprised to find that they are rhodopsin–porphyropsin mixtures.

Bridges suggests that we found no difference between the visual pigments of the dorsal and ventral parts of the retina possibly due to a change in the rhodopsin–porphyropsin ratio caused by keeping the fish in artificial light. Our value of the mean λ_{max} of the rods is, however, within 1 nm of the value he and Schwanzara[1] obtained for extracts of the rod pigment, inferring that the rhodopsin–porphyropsin ratio in the rods we measured in both regions of the retina was the same as that of their extracts. If *Anableps* does have higher proportions of porphyropsin in the dorsal retina in its natural habitat, we would have expected the λ_{max} of Bridges' extract to have been longer than our rod mean λ_{max}. Unfortunately, extracts normally contain only rod pigments and give no information about the distribution of pigments across the retina or the nature of the cone pigments. Thus, further microspectrophotometric studies are required.

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1 Schwanzara, S A. *Vision Res* **7**, 121–148 (1967)
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BOOK REVIEWS

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Chemical Engineering
Chemical Technology
Plastics & Rubber Technology

Multiple realities?

Marie Jahoda

Einstein's Space and Van Gogh's Sky: Physical Reality and Beyond. By Lawrence LeShan and Henry Margenau. Pp.268. ISBN 0-02-570460-5. (Macmillan, New York: 1982.) $14.95. To be published in the United Kingdom in January 1983 by Harvester.

IN THE last quarter of the nineteenth century psychologists, impressed by the advances in the hard sciences, emulated their big brother and issued a unilateral declaration of independence from philosophy. Both, emulation and independence, were powerful motivators; they appeared to work reasonably well in mainstream psychology, even though doubts about the wisdom of either were raised from the beginning.

For a good part of the twentieth century emulation was based on the mechanistic physics of earlier periods. But physics had undergone revolutionary changes. Its new concepts — particularly Heisenberg's principle of uncertainty and the probabilistic nature of knowledge in many areas — gradually permeated the intellectual culture of psychologists, many of whom were all too aware of uncertainties in their methods, concepts and findings. References to Heisenberg increased in the psychological literature, used defensively however, rather than as a stepping stone to further advances, as was the case in physics.

The book under review, written by a research psychologist and a physicist, repeals the declaration of independence from philosophy in both disciplines and searches for a redefinition of their communalities and differences. It is organized in three parts, entitled "The Meanings of Reality", "The Search for Scientific Truth" and "Domains of the Social Sciences". Philosophers of science have dealt with such issues often as outsiders to the practice of science and have, as a rule, concentrated on the hard sciences; the reversed intrusion by practitioners of science into epistemology is largely limited to a specific field. The increase in books, of which this is one, that transcend both these limitations is a welcome sign of efforts to put the human sciences on a more solid base, even those aspects where matters count that cannot be counted; at least not yet.

In crude outline (omitting many interesting asides such as Heisenberg's appreciative comments on Goethe's theory of colours) the rather involved argument of the authors runs thus: the materialistic concept of reality has been shown to be limited by "the works of Heisenberg, Schrödinger, Einstein, Born, Freud, and Jung" (p.1) and needs reformulation. While common sense still regards as real only the external world that can be seen or touched, modern physics constructs as real events in the macro- and in the microcosm that are beyond the sensory competence of the human organism and can be understood only mathematically or through inference from observeable consequences. Newton's formulation of reality was not wrong but insufficient in view of later constructions that left room for his but transcended it. In particle physics and astrophysics visualization is no longer possible, but every physicist regards the regularities established in these areas as real. New constructs of reality have been invented and their correspondence to observation has been discovered.

Physics was for a time erroneously believed by some human scientists to have solved their problems. This is wrong. The aspect to be taken on board from modern physics is the idea that it is legitimate and, for certain areas, necessary to use alternate interpretations of reality, particularly when psychologists go beyond overt behaviour to study the domains of inner life. Here it is unavoidable to assume that purpose and intention are real, even though they can only be inferred (much like the electron), not seen or felt by an observer. This is a construction of psychic reality with which many psychologists have been familiar since the beginning of the century in a systematic way, but even St Augustine knew that psychological time had to be construed differently from measureable time, and accepted both as real.

Just as physics distinguishes between various domains (mechanics, quantum physics and so on) that are based on different constructions of reality, so the human sciences must identify their domains. Those suggested here in addition to molar behaviour are: art, parapsychology, ethics and consciousness. A curious assembly.

Ignoring this idiosyncratic choice of domains, what is one to make of all this? As a non-physicist I learn from this and other expositions of modern physics for the layman one important lesson: I have to take it on credit. As a non-philosopher I can only marvel at the amount of mental energy that has been expended over the centuries on pondering what reality really is: a dream, a unitary or multiple cosmos, a deliberate creation or an evolution by chance and necessity? These eternal ontological questions are metaphysics, not science. As a psychologist, I agree with the authors of this volume, that, whatever one's metaphysical stance ". . . we can never determine the 'true' shape and order of reality. We shall have to give up this dream" (p.24). All we can do *qua* scientists is to invent different ways of thinking about reality (including consciousness as much as the physical world) and check the extent to which concepts correspond to observation.

Two pages later, on p.26, the various constructs have changed into various essences, when the phrase is ". . . multiple, equally valid realities . . .", not constructs of reality. The very chapter title "Alternate Realities" implies the same deviation from p.24, and throughout the book there are other examples of confounding what there is with how we think about it. Surely we must assume that particles performed their indeterminate dance even before modern physics began to look at them as real?

It may seem pedantic to criticize the authors for what might be regarded only as elliptic phrasing, given that the basic position of an unending asymptotic approach to reality was stated clearly early on. But this is not the only case of loose talk about concepts crucial to the entire argument. Another is the concept of purpose, an indispensible concept in much of psychology where it is regarded as a state of consciousness in the here and now. On p.132, however, it is equated with "determination by the future"; on p.157 "future states influence present occurrences". This is magic. Once again, St Augustine and modern psychology know better. It is the present anticipation, not a future event which may or may not occur, that influences experience and action.

There are also some minor flaws: R.F. Bales is consistently misspelled; the index is not very helpful; some of the down-to-earth examples are not convincing; and to usurp art and ethics for psychology seems to me misplaced imperialism. Altogether, an interesting intention has been disappointingly executed. □

Marie Jahoda is Professor Emeritus of Social Psychology and Consultant to the Science Policy Research Unit at the University of Sussex.

What's what in organic chemistry

E.R.H. Jones

Heilbron's Dictionary of Organic Compounds, 5th Edn. Executive editor J. Buckingham. Seven volumes, pp.7,848. ISBN 0-412-17000-0. (Chapman & Hall/Methuen: 1982.) £975, $1,950.

THE number of compounds described in the literature provides a more precise measure of the growth of chemistry than publications or other indicators. Organic chemistry, thanks to the tetravalency of carbon and the stability of its incestuous bonds, is responsible for most of these compounds; even the recent plethora of inorganic compounds is attributable to organic coordinating components. The number is now nearly five million and, resulting from advances in techniques, many recent additions have large and complicated structures. Remarkably, however, this situation is being coped with.

Some 50 years ago Professor Ian Heilbron and H.M. Bunbury recognized the need of chemists for something akin to the *Concise Oxford*, and the first edition of the *Dictionary of Organic Compounds* (1934), a most successful venture, provided a readily accessible source of information. "Heilbron" is the first point of enquiry for many, and this fifth edition (the fourth appeared in 1965) is timely indeed. The need for facile reference to the literature has never been greater.

In the new edition the original policy of encompassing all of interest and importance is more specifically defined; the intention has been to include fundamental compounds of simple structure, the majority of important natural products, compounds of commercial value, and laboratory chemicals and those with special chemical, physical or biological properties. Selection of the 50,000 entries, by deletion as well as addition, which overall results in the mention of about 1 in 30 of known compounds, has been satisfactorily achieved with the assistance of a hundred chemists, including some 20 special editors, all experts.

With this highly organized team and using modern methods of data handling, the selection and compilation of information and production of the books were completed within five years so that the primary literature has been covered to the end of 1980. Although the new edition involves "the most radical revision and modernisation", the traditional format, the generous provision of structural formulae and indeed all the familiar features have been retained. And there are significant changes. Citing only one author's name is a sensible economy and specifying the content of references (e.g., isol, synth, struct, cmr and so on) is a valuable innovation, as is the extensive inclusion of toxicity and hazard indications and references.

Organic chemists think in terms of structural formulae and need assistance to translate these into dictionary entries. Help is abundantly available from the molecular formulae and name indexes. Alternative and recommended names are given, indication of their sources is useful and Chemical Abstracts Registry Numbers provide a nomenclature-independent link to, and from, those printed here and to the other services which are increasingly using Registry Numbers. A heteroatom index, in 62 sections, points to the entries containing elements other than C, H, O, N and halogens. Thus we find amongst the 1,500 organo-metallic compounds 19 of copper and 25 of manganese. Accessibility to the alphabetical entries from the indexes is enormously facilitated by serial numbers for each entry, and will be most helpful with the annual supplements (the first promised for 1983).

By comparison with the cost of periodicals and other books, the seven volumes and 7,800 pages represent excellent value for scarce library money. The *Dictionary* is an essential adjunct wherever organic compounds are used, nowhere more so than in educational establishments where students need training to cope with the population explosion in organic chemistry. □

Sir Ewart Jones is Emeritus Professor of Chemistry at the University of Oxford.

The bones of palaeopathology

D.R. Brothwell

Identification of Pathological Conditions in Human Skeletal Remains. By Donald J. Ortner and Walter G.J. Putschar. Pp.479. (Smithsonian Institution Press/US Government Printing Office: 1982.) Pbk $12.

TWENTY-five years ago, as a young graduate of University College London, I was puzzled and impressed by the near total eclipse of interest in sub-fossil skeletal remains, and especially the evidence of palaeopathology which could be found in them. A quarter of a century earlier, in Egypt and eventually at the same college, Sir Grafton Elliot Smith and colleagues had produced distinguished studies on thousands of skeletons and mummies, paying close attention to the evidence of disease. Yet in my time, bones were a matter of considering the many scraps of fossil hominids or were simply used in the general teaching of comparative vertebrate anatomy.

The balance of interests in the biology of man is ever changing, however, with the result that human disease ecology and even such highly specialized topics as the problems of identifying disease in excavated skeletal remains is receiving renewed attention. Evidence of this, if evidence were needed, is this handsome large-format book by two of the senior names in palaeopathology.

Considering the vast literature on medical diagnosis, one might imagine that there were sufficient references to the differential diagnosis of skeletal disease. What had Elliot Smith, Ruffer, Wood Jones and all the other golden oldies used? The answer is that they made do with rather limited texts and similarly limited dry bone specimens in medical museums, and with a range of clinical experience of bone pathology in those pre-antibiotic times. But museum displays are changing (if not shrinking) and dry bones are no longer priority exhibits. It was thus becoming critical that some detailed stock-taking was undertaken, preferably with an eventual published text and "atlas" of good illustrations in mind.

These changes then, and the growing literature on bone pathology, demanded an up-to-date review of the subject, preferably with a large range of well-selected, high-quality illustrations. Although a number of introductory texts and two symposium proceedings appeared in the 1960s, the definitive reference work of the kind provided by Ortner and Putschar remained a much needed project. This is a well-illustrated, carefully researched collaborative effort providing a comprehensive review of pathological conditions that affect the human skeleton. Having seen the authors in action, scanning the Royal College of Surgeons collection in London, I can vouch for the care with which they have not only surveyed past literature, but personally examined a very wide range of specimens of known diagnosis. There are thus authoritative details of a wide range of diseases affecting the skeleton, from congenital abnormality, trauma and arthropathy, to endocrine and metabolic disturbances, circulatory disorders and the numerous divisions of infectious disease (including less well known bone pathology, the result of mycotic infections, brucellosis, sarcoidosis and echinococcosis).

There is no doubt that this volume is an essential reference work for all those who handle ancient human skeletal material. It should also hold interest for medical historians as well as radiologists, pathologists and orthopaedic specialists. □

Don Brothwell is Reader in Zooarchaeology at the Institute of Archaeology, London.

Plants and biochemical defence

John Friend

Phytoalexins. Edited by John A. Bailey and John W. Mansfield. Pp.334. ISBN 0-216-91162-1. (Blackie/Halsted: 1982.) £28, $79.95.

THE existence of phytoalexins as antifungal compounds actively accumulated by plants was first proposed in 1940 to explain the response of potato tuber tissue to inoculation with avirulent and virulent races of *Phytophthora infestans.* Although the major sesquiterpenoid phytoalexin from potato was not isolated until 1968, in the intervening period phenolic and terpenoid phytoalexins were identified in orchids, sweet potato, carrots, peas and French beans. Many more plants have since been investigated for their ability to produce phytoalexins in response to environmental stress, as well as to challenge not only by fungi by also by bacteria and viruses and by salts of heavy metals.

The aim of the editors of *Phytoalexins* was to review in detail the compass of current research into these compounds; with the aid of a battery of international authorities on the various topics, this is what they have done and excellently so. Broadly the book falls into two parts, the first of which deals mainly with the chemical study of phytoalexins.

Since the majority of such compounds have been isolated from the Leguminosae and the Solanaceae a separate chapter is devoted to each of these families (by J.L. Ingham and J. Kuć, respectively), whereas the phytoalexins from all the other families are covered in a further contribution by D.T. Coxon. Ingham's chapter is particularly valuable in that it includes chromatographic and spectroscopic data which can be used for the separation and identification of phytoalexins. In his comprehensive review of phytoalexin biosynthesis A. Stoessl separates the phytoalexins according to their biosynthetic origin from shikimic acid, acetate-polymalonate and mevalonate; he also emphasizes the value of stressed tissue for biosynthetic studies.

In the remaining contributions, comprising the second part of the book, the accent is on the possible roles of phytoalexins in plant diseases. The reactions involved in the metabolism of phytoalexins both by microorganisms and plants are described by H.D. VanEtten and colleagues, and D.A. Smith discusses the toxicity of phytoalexins and their metabolites. J.W. Mansfield then provides a detailed description of phytoalexin accumulation in the interaction of several leguminous and solanaceous plants with fungi, bacteria, viruses and nematodes. It is clear from these accounts that pathogenicity cannot necessarily be explained by the ability of the invading pathogen to tolerate or metabolize phytoalexins, although in many cases of resistance there is sufficient accumulation of phytoalexins to account for this resistance; nevertheless other factors such as suberization and lignification must be taken into account.

In his review of the mechanisms of phytoalexin accumulation, J.A. Bailey indicates that the underlying mechanisms leading to the build-up of all classes of phytoalexin may be similar. He proposes that host cell death releases an endogenous elicitor which triggers the enzymes involved in phytoalexin biosynthesis, and points out that specificity would involve control of cell death. The final chapter, written by the two editors themselves, is an excellent summary of work on phytoalexin function to date and of possible ways in which it could proceed in the future.

The overall presentation, particularly of the formulae and diagrams, is good and there are very few errors. Moreover there is a comprehensive bibliography with each chapter, and a compound and an organism index at the end of the book (though, unfortunately, no author index). *Phytoalexins* will be the standard work on the subject for some considerable time; as such it is an essential reference volume for all workers in plant disease physiology and phytoalexin biosynthesis. ☐

John Friend is Professor and Head of the Department of Plant Biology at the University of Hull.

Flies in the lab

Michael Ashburner

A Handbook of Drosophila Development. Edited by Robert Ransom. Pp.289. Hbk ISBN 0-444-80366-1; pbk ISBN 0-444-80418-8. (Elsevier Biomedical: 1982.) Hbk Dfl.250, $116; pbk Dfl.100, $46.

THE EDITOR of this collection of reviews on the development of *Drosophila* states his objective as being:

> to provide a useful and practical research aid, which may be kept close at hand on the researcher or students [*sic*] own bookshelf.

This, however, is not a "handbook" in the German tradition; the fact that you can actually pick it up with one hand establishes that. Yet its chapters do provide useful, if not over-scholarly, reviews of the embryonic and imaginal development and structure of *Drosophila.* There is, of course, some overlap with Vol.2 of *The Genetics and Biology of Drosophila* (Academic, 1978) — a work in which I must declare some interest — but except for two

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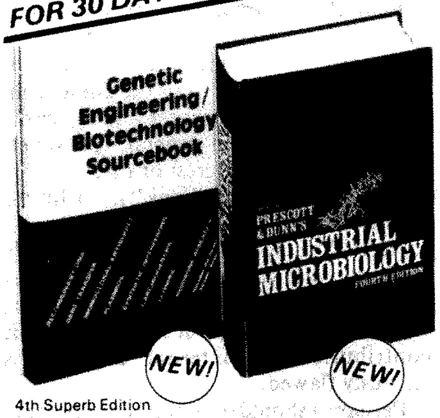

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chapters this is minimized by the differences in organization between the two works. I am disappointed, however, that this book has not been truer to its design, restricting itself to hard data and not degrading this information with speculative "noise". In each chapter the worst sections are those with some such heading as "Genetic studies on . . . ". These contribute very little to what is already easily available and are, in general, too superficial to be of any interest or use.

An editor of a "handbook" has a duty to ensure that the factual information is correct. This ideal can never be wholly achieved, yet I cannot but warn the reader of this book that the editor's own chapter, on techniques (and to a lesser extent his contribution on internal organs), is seriously flawed.

Perhaps I should quote one or two errors of fact: "Each female only mates once". Quite untrue. "Sex is determined by X chromosome dosage". Again untrue, it is determined (in *Drosophila*) by the X:autosome ratio. "This phenomenon [mitotic recombination] can be induced spontaneously". No comment.

Finally, I defy any reader to understand the mechanism of mitotic recombination from Fig.6, which shows undivided centromeres as if this were the first division of meiosis; I also do not approve of the illustration of methods to screen for mutations by specialized, rather than more general techniques (i.e. Figs 11 and 12).

Happily, this standard is not maintained in the other chapters. There are one or two annoying features (for example Fig.1 of Chapter 4 and Fig.16 of Chapter 7 are identical), but in general the central eight chapters are solid and competent and make the book a useful addition to the *Drosophila* biologist's laboratory bookshelf. ☐

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Twenty-five years of cyclic nucleotides

D.G. Hardie

Cyclic Nucleotides, Vols 1 and 2. Edited by J.W. Kebabian and J.A. Nathanson. Vol.1 *Biochemistry*, pp.557, ISBN 3-540-10786-X; Vol.2 *Physiology and Pharmacology*, pp.888, ISBN 3-540-11239-1. (Springer-Verlag: 1982.) Vol.1 DM390, $173.20; Vol.2 DM 560, $248.70.

IT HAS been possible to show that the response of the homogenates to the hormones occurred in two stages. In the first stage, a particulate fraction of homogenates produced a heat-stable factor in the presence of the hormones; in the second stage, this factor stimulated the formation of liver phosphorylase in supernatant fractions of homogenates in which the hormones themselves were inactive [T.W. Rall, E.W. Sutherland and J. Berthet *J. Biol. Chem.* **224**, 463–475, 1957].

This unpretentious announcement of the discovery of a heat-stable factor, later identified as cyclic AMP, quietly heralded the era of the cyclic nucleotides. While the authors no doubt realized that their observations represented a breakthrough, they can hardly have guessed at the impact they would ultimately have. Twenty-five years later it is clear that cyclic AMP has a central role in cellular regulation, equivalent to that of its parent nucleotide ATP in metabolism.

- A second edition of *Germ Cells and Fertilization*, Book 1 in the series *Reproduction in Mammals*, has recently been published by Cambridge University Press. Prices are hbk £15, $24.50, pbk £5.95, $11.95.

Four further volumes in the second edition of the series (which is edited by C.R. Austin and R.V. Short) are scheduled to appear in due course.

These two volumes provide the latest review of the field and are, in the words of the editors, an attempt at a "comprehensive and up-to-date anthology" with contributions by 72 well-known cyclic nucleotide researchers. The books appear in the much-respected *Handbook of Experimental Pharmacology* series, to which all pharmacology departments no doubt subscribe. But do they deserve a wider audience?

The two volumes are to a certain extent independent of each other. Volume 1, *Biochemistry*, covers the enzymology of cyclic nucleotide metabolism and of the protein kinases which form the only known intracellular receptors for the cyclic nucleotides. It also provides ample discussion of the interactions between cyclic nucleotides and that other ubiquitous cellular regulator, the Ca^{2+} ion; calcium, at least in its free cytosolic version, seems now to have almost acquired the status of "honorary cyclic nucleotide". Volume 1 gives a well-balanced and reasonably up-to-date account of these aspects of the field. A notable omission, however, is any discussion of protein phosphatases, which do not even appear in the index. This is regrettable because it is now apparent that some actions of cyclic AMP are mediated through inhibition of protein phosphatase-1 by the heat-stable inhibitor protein which is phosphorylated by cyclic AMP-dependent protein kinase (a topic reviewed recently by Philip Cohen in *Nature* **296**, 613–620; 1982).

Volume 2, *Physiology and Pharmacology*, covers, as it were, the applications of cyclic nucleotides in physiological processes. It gives an idea of their breadth of function, with topics ranging from slime moulds to the mammalian brain.

An interesting contrast emerges from these volumes between our knowledge of the role of cyclic AMP and that of cyclic GMP. Although cyclic GMP was discovered as long ago as 1963, its functions are still largely obscure. The Yin–Yang hypothesis, so delightfully named by Nelson Goldberg, in which cyclic GMP was seen as a messenger opposing the effects of cyclic AMP, now seems untenable. While Murad speculates here that cyclic GMP might be a general feedback regulator of redox state, it seems possible that despite its ubiquitous occurrence it in fact performs only specialized functions (for example in dark adaptation in the retina as discussed by Farber in Vol.II). This would be rather analogous to its parent nucleotide GTP which, in its guise as energy donor rather than nucleic acid precursor, has a small number of rather specialized roles one of which is the regulation of cyclic AMP formation by adenylate cyclase.

In his introduction to the two volumes, Rall states that the 1960s saw a great advance in our understanding of the mechanism of action of cyclic AMP in physiological systems, while in the 1970s the progress has been marginal. This is indeed the overall impression given by Vol.II, with a very few of the systems described being understood in molecular terms. However I believe this view to be misleading. It reflects, in part, an emphasis on systems with relevance to pharmacology where the basic processes themselves are not understood. It also ignores the fact that of the 20 or so physiological substrates for cyclic AMP-dependent protein kinase whose function is known, all but three have been identified since 1970. Given that these substrates are tabulated in the chapter on cyclic AMP-dependent protein kinases in Vol.1, some of the omissions in Vol.2 are very surprising. For example, a chapter on "Regulation of Lipid Metabolism by Cyclic Nucleotides" makes no mention of any of the well-characterized phosphoproteins involved in lipid synthesis, such as acetyl-CoA carboxylase or hydroxymethylglutaryl reductase. And that entitled "The Role of Cyclic Nucleotides in the Vasculature" states that the causal link between cyclic AMP and the relaxation of vascular smooth muscle is unknown — the work of Adelstein and others on relaxation of smooth muscle via phosphorylation of myosin light-chain kinase is not mentioned. Readers who prefer their explanations in molecular terms, or at least in terms of defined proteins, will find Vol.II disappointing, and in my view this need not have been the case. ☐

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Where not to put strategic missiles

The Reagan-Weinberger solution to the MX-siting problem is ingenious, probably unworkable and certain to confuse the generals. It points only towards the need for strategic arms control.

The Reagan Administration appears to be capable of thinking not merely the unthinkable but the unworkable. Now, after years of uncertainty, Mr Caspar Weinberger, the US Secretary of Defense, has proposed and President Ronald Reagan has agreed that 100 MX missiles should be based in a narrow tract of land in Wyoming, handy for the short journey over the North Pole to targets in the Soviet Union but also so well advertised in position as to be itself an easy target for Soviet intercontinental missiles. In the bewildering spectrum of solutions to the problem of how the MX missiles should be sited, the Reagan-Weinberger solution is at the other extreme from that to which President Carter appeared to be most attracted — building underground railway systems so that 100 missiles could be distributed more or less at random (so far as outsiders could tell) between more than 2,000 potential launching silos. The Carter solution to the problem of where and how to put the missiles had the obvious advantage that a potential enemy of the United States (supposed for these purposes to be the Soviet Union) could make an effective first strike against the US stock of strategic missiles only by making 2,000 separate attacks on as many holes in the ground, of which fewer than five per cent would be occupied by missiles. The disadvantage, as the people of the western states told Mr Carter clearly enough, was that the whole arrangement would be an infernal nuisance, socially as well as environmentally. His taxpayers were also understandably alarmed at the cost. The Reagan-Weinberger solution has the advantage of avoiding the most obvious difficulties. Its disadvantage, which influential people as different as Professor Charles Townes (see *Nature* 18 November, p.208) and Dr Richard Garwin are pointing out, is that it lacks credibility.

Although intercontinental ballistic missiles are only a little more than a quarter of a century old, they have been with us long enough for the question of where to put them to be recognized as important, crucial and vital (in the sense of life and death). At the outset, when missiles were so inaccurate that their only accessible targets had to be large cities, and when the chance of a missile silo being hit by an attacking missile aimed at it was virtually zero, superpowers could base their intercontinental missiles in silos whose locations were known to potential enemies without fearing that their capacity to retaliate would be impaired by a preemptive strike. For the past decade, however, and especially with the development of techniques for guiding warheads to their targets without continual reference to the launching site (i.e. intelligently), that calculation has gone by the board. The chances now are that it is possible for one side or the other to destroy point targets on the other side with weapons whose yield in terms of the equivalent in conventional explosives is no more than, say, 100,000 tonnes. So the United States is rightly worried that its force of nearly 1,000 Minutemen scattered through the western states has become vulnerable to a first strike, and anxious that it should be replaced by something better or less vulnerable. Even those who disdain entirely the basis on which such calculations are made must acknowledge that a superpower worried about the viability of its striking force is a dangerous superpower, likely to be tempted to fire them off before there is an undeniable need to do so. In other words, so long as superpowers exist, they must be helped to feel confident about the security of their missile forces for otherwise they will become trigger-happy and dangers to the rest of us.

The principal Soviet striking force is no better placed. While missile launchers are able to shuttle along most of the length of the trans-Siberian railway and thus secure a measure of protection from uncertainty, the speed with which they move (slow) and the steady improvement of satellite reconnaissance also makes them vulnerable to a first strike from some other superpower. So there is a case for hoping that both of those superpowers with a legitimate interest in the maintenance of counter-force missile flights should be helped towards a system for their deployment that will be more credible for the generals, more conducive to good nights' sleep by generals' political bosses (and less burdensome for taxpayers). What can this be?

The Reagan-Weinberger solution in Wyoming is a reasonable starting point. The calculation is that a hostile superpower would first attack the southernmost missile silos; otherwise succeeding waves of missiles would necessarily have to travel through electrically and mechanically disturbed atmosphere. So why not build the southernmost silos, but not put missiles in them? With a little luck, it might be possible to get by with only half the force of 100 missiles now planned. But with a little ingenuity, it will be possible to save still more public money and at the same time to reinforce the now shaken confidence of the generals, by leaving all the silos empty but deciding, secretly, to put a few MX missiles in existing holes in the ground, the Minuteman silos soon to be vacated for example. Or it could, with an even greater exercise of ingenuity and intelligence, be recognized by both the superpowers that what the Reagan-Weinberger solution of the MX problem demonstrates is the need for both of them to opt out of the application of a first strike capability.

In retrospect, the 1950s, when the two superpowers were able only to threaten each other with the crudest forms of annihilation, were comparatively comforting times. The gulf between conventional conflict and mutual nuclear annihilation was huge — so huge that nobody ever thought it would be bridged. What technology has made possible, in the MX missile and its Soviet counterparts, is the prospect that the other fellow's strategic force might be destroyed before it can be used, coupled with a haunting anxiety that the missiles that will accomplish that cannot themselves be hidden or protected. The sensible course, therefore, is to agree to give them up, by negotiation at Geneva. For the time being, neither superpower need abandon its force of submarine-launched missiles, the reason why each can say to the other that annihilation of people, not machines, is still within its grasp. But the Reagan-Weinberger solution should, by the fancifulness of its disposition of expensive hardware, point urgently in that direction.

The Reagan-Weinberger solution of this dilemma is ingenious and even daring but may not create the sense of confidence that both generals and taxpayers in the United States are likely to demand. The theory, of course, is simple. If a hostile warhead should attack some missile silo in the proposed "Dense Pack" missile park in Wyoming, destroying that and putting its immediate neighbours out of action, the calculation is that there would still be others (further north?) still capable of reacting, and that these would be invulnerable to attacking warheads because they would be protected by the atmospheric ionization (radio blackout) and turbulence caused by the first attacks. The underlying assumption, of course, is that the attacking warheads

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would be fashioned as those intended to be packed (ten to a rocket) in the MX missile are designed. The trouble is, for the Reagan-Weinberger solution, that attacking warheads might be differently designed, made so as to make large holes in the ground. But in any case, what the generals in charge of the proposed missile park in Wyoming will know up until the last moment is that there has not and will not have been a practical demonstration of the doctrine of fratricide, the argument that the first attacking warhead will prevent others from doing their job. And in the event, uncertainty in a general's mind is more important than any amount of theoretical calculation by professional people who have studied the effects of nuclear weapons on the atmosphere. The calculations may be right, but if the generals do not accept their implications, they may ask the president of their day (unlikely to be Mr Ronald Reagan) to press the button that will prevent a preemptive attack. And he (or she) will have no choice but to agree.

Tell us about our friends

The President of the Royal Society has made a brave speech on Soviet illiberality.

How should a learned society, or for that matter a national rugby football union, conduct international relations with its counterparts in countries whose governments command little respect? This old dilemma is too little discussed, but Sir Andrew Huxley seems to have enlivened this year's anniversary meeting of the Royal Society (see next page) by an account of how deliberate and even obdurate innocence can triumph where diplomacy dictates mere compromise.

Conventional logic goes like this. By convention, (and United Nations charter) governments do not interfere in each others' internal affairs except in those mercifully rare circumstances in which they go to war with each other. National academies (not to mention rugby football unions) are even less well-equipped to effect political change in any sphere. Their analysis of what needs changing, and how, is certain to be less expert than that of the political professionals, diplomats on the international stage. Their assessment of how change might be accomplished is bound to be amateurish. Their resolution to carry through a recipe for change is bound to be compromised by differences of opinion among their members. This is why the Royal Society has been right, in this presidency and the last (Lord Todd's), to insist that the character of the Soviet government was not part of its concern, and was in any case a question outside its competence. If Soviet citizens are locked up or harassed in other ways because Soviet laws expect and exact more from them than do other people's laws, the fact that those injured happen to be scientists (or rugby footballers) is strictly speaking irrelevant. Indeed, when the going is rough, conventional opinion has it that the preservation of close relationships is all the more important. This is the spirit in which Huxley this week insisted that the pitiful trickle of professional exchanges with the Soviet Union should not be compromised by his decision to complain in public about the Soviet academy's apparently deliberate misunderstanding of questions about the ill-treatment of certain Soviet scientists.

Huxley's predecessor, Lord Todd, maddened much of the British scientific community by his proper repetition of the doctrine that sovereign governments of any kind are autonomous within their legal territory, but then won unexpected friends with his fierce attack on Soviet oppression of scientists at the Hamburg meeting on the Helsinki accords three years ago. Huxley's line has been more guileful. Enquire about your friends. When challenged, put the details down on paper. Then hope for the best but anticipate the worst. And when the weasel answer comes that Sakharov has been dealt with leniently, and when you know that he is still confined in Gork'ii with the third winter closing in, simply say in public that the explanation is insufficient. The doctrine of the autonomy of sovereign states is an essential foundation of orderliness, and should not be undermined, but what is to prevent us asking after our friends?

Adam Smith forgotten

This week's meeting on international trade could have been disastrous but should have been better.

In the nine years since the last formal meeting of the governments that are party to the General Agreement on Tariffs and Trade (GATT), the world for which the agreement was originally negotiated has been transformed out of recognition. Nine years ago, the great increase of the international price of oil had only just begun, and few could guess what the consequences would be. Now, of course, we know: inflation and recession in the industrialized world, inflation and stagnation elsewhere. The rich countries are at best marking time, (but Japan, ironically classified at GATT as a country in the process of industrialization, is an exception); the poor are mostly becoming poorer (but countries such as India may yet be exceptions). The barely hidden agenda for this year's meeting seems to have been the wish of industrialized countries to look each other in the eye so as to discover whether they should hold to the principles of free trade on which GATT is founded (and if so whether their partners could be persuaded to obey the rules) or whether they should follow the politically more prudent course and settle for protectionism instead. Although Monday's formal communique reaffirms the principle that free trade is best, only in the months ahead will it be clear how many of the signatories believe that to be the case. (The government of Australia walked out at the weekend on the grounds that the then promised declaration was too leaky a commitment, generously studded as it was with phrases such as "wherever practicable".)

The trouble with GATT, like any other club, is that its members can and will resign whenever they think the costs of membership outweigh the benefits. And the trouble with a club whose members agree to trade freely with each other is that the costs are the more easily perceived. Most industrialized countries, now struggling with unpalatable and perhaps even unsupportable unemployment, know that measures to keep out imports would have an immediately beneficial effect. Domestic customers now buying efficiently-made goods imported from abroad would instead buy less efficiently manufactured domestic products, creating jobs for fellow-countrymen. Only in the longer run would the snags show up — customers required to support inefficient industries are on that account less prosperous than they might be. Two centuries ago, Adam Smith made the point quite simply: the wealth of nations stems from their capacity to bring about a rational division of labour, domestically and internationally.

This time round, GATT has merely stayed still. The constructive parts of the communique on Monday are those referring to studies to be carried out in the first instance by officials on some of the more contentious issues — should free trade extend not merely to manufactured goods but to invisibles — insurance, shipping and the like? (Yes, whatever Japan says.) Should free trade also extend to agriculture? (Yes, even though the European Community is outwardly united in its opposition.) What should be done about counterfeiting? (It should be penalized.) And the plight of countries in the process of industrialization? Who knows? If GATT hopes to survive, it must answer these questions before too many of its members drift away.

Meanwhile, Adam Smith is an evocative reminder that a rational (market-driven) division of labour cannot be accomplished without upheaval. Even in the best-regulated international communities, in the short run one country's industrial gain is likely to be another's loss. In the heady 1960s, when it seemed as if economic growth (and world trade) would continue without interruption, there was endless talk in places such as GATT of the virtues of adjustment assistance — ways of compensating losers for short-term losses. But that talk has now faded. Yet when the danger that nations will barricade themselves behind their trade barriers is greater than ever, the need for such assistance is ironically more urgent than it has ever been.

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London and Moscow in hassle

Huxley attacks Soviets on civil rights

A complaint by the Royal Society about the treatment of Soviet scientists by their government has been delivered to the Academy of Sciences of the USSR, and the reply has fallen "far short" of what was promised, and hoped for. This was the most arresting message in the anniversary address to the Royal Society, delivered by Sir Andrew Huxley, the president, last Tuesday. While Sir Andrew reaffirmed his adherence to the society's view that scientists who break their countries' laws are just as liable to punishment on that account as are other citizens, his decision to make public the Royal Society's dissatisfaction with its enquiries of Soviet scientists is a break with precedent and recent practice.

The exchange between the two academies apparently began in May this year, when a Soviet delegation visited the United Kingdom. Sir Andrew explained this week that while not wishing "to sacrifice international cooperation between individual scientists in the hope of persuading the government of another country to change its internal policies", oppressive treatment of scientists by the Soviet government was already "harming scientific interchange" with Britain.

By way of illustration, Sir Andrew said that the funds available each year for exchanges with the Soviet Union are not fully taken up, partly because British scientists are unwilling "to spend time in the USSR because of the oppression in that country of dissident scientists, the refusal of permission to Jewish scientists to emigrate to Israel and recently the revoking of the academic degrees of certain individuals".

The meeting with the Soviet delegation in May this year seems to have been turbulent. Sir Andrew said this week that "we took this opportunity of putting this point forcefully to them". By his account, the Soviet delegation, emphasizing that the Academy of Sciences was not responsible, offered to investigate any cases the Royal Society cared to specify in detail, and to report. The Royal Society then apparently wrote with details of twenty-one Soviet scientists in trouble — seventeen who were being "subjected to various forms of punishment", five refusniks and three who had been prevented from attending scientific meetings in the United Kingdom. The reply from Moscow appears to have arrived only early in November, and to have been unsatisfactory. "Seventeen of the twenty-one scientists named in our letter are not even mentioned, and I take this as a tacit admission that in most or all of these cases there is no respectable excuse that can be put forward as justification of the treatment they have been given."

Huxley went on to say that he finds it "difficult to accept" the explanation given for the treatment of Alekssander Lerner, who has been refused permission to emigrate to Israel; can "reasons of national security, including knowledge of classified and defence material", apply to one dismissed from his post at the institute of control sciences in Moscow eleven years ago? The Soviet academy also says that V. Kislik is imprisoned for "petty crimes" without saying why he has been deprived of his degrees, and that Y. Orlov is imprisoned for having damaged the Soviet Union politically.

The case of Andrei Sakharov seems, however, to have been the most serious bone of contention. Sir Andrew said that the Royal Society's letter complained that Sakharov was being "persecuted for his work in defence of human rights", and went on to say that nobody denied that he had criticized restrictions of individual freedom in the Soviet Union, published writing abroad and yet been allowed to remain a member of the Soviet academy.

The Soviet academy's reply seems to have been a deliberate misreading of the complaint. Sakharov's exile to Gork'ii is said to be "comparatively mild" in relation to "his actions, which had the aspect of being crimes against the state". Huxley says that the Soviet reply on Sakharov emphasized his dissent from Soviet policies on arms control whereas "most in the West" consider that the "real reason" for his exile is his criticims of "the illiberal internal regime in the USSR".

Huxley concluded his address by saying that his decision "to express publicly my own feelings" is to be taken as a definition of his position, but that he would not wish to curtail the exchange of scientists between the two countries or restrict visas "to *bona fide* scientists wishing to attend international meetings". ●

French university lecturers protest

Hundreds of French university lecturers marched from the Sorbonne in the centre of Paris to the ministry of national education last Friday, in protest against what they see as a complete abandonment by the minister, M. Alain Savary of any serious — and socialist — reform of the university system.

Much had been — and is — expected of Savary's "law for higher education" to be put before parliament by the end of the year, but the higher education trades unions have not been much impressed so far. Hence the strike.

Backing for the union view, however, may not be particularly strong. A few hundred demonstrators are only a small part of the 44,000-strong community of university teachers, and the union demands for a single kind of lecturer (including professor), with promotion by seniority are seen as unrealistic in many quarters.

Nevertheless the outburst of grievances among the unions has a substantial basis — the enormous blockage of posts within the universities, which according to ministry calculations will see only 158 retirements next year, one third of one per cent of the staff. Thus a common international problem among universities is gravely exaggerated in France, because of a peak in the recruitment of staff in the late 1960s, before and after the "troubles" of 1968.

Moreover, this tinder-box situation has been aggravated by some expectation of relief, after the savage activities of the previous, Giscardian, minister of the universities. But Savary is offering little or no relief, and indeed is piling on the pressure with a reduction in part-time teaching allocations, a lengthening of the university year, and an insistence that since university staff are civil servants, they must take no more than five weeks' holiday. The number of students would also increase from its present 800,000 to 1 million, said the minister.

Recently the minister further announced to the unions that their dream of a single category of lecturers was unattainable, and that there would instead be a system much like the present, with effectively lecturers, senior lecturers and professors with appointments between grades determined by peer review. That was the last straw, and led the left-wing unions to the strike.

Now, however, the ministry is suggesting that "new propositions" will be made within a couple of weeks — apparently opening all options once more.

This seems to leave the preparation for a new higher education law, one to replace the somewhat rushed law of 1968, in an utter shambles. So many interest groups are at work that it is little wonder that the French education system is sometimes described as the largest political system outside the Red Army.

Moreover, there is a further muddle ahead: the question of appointments and promotions for 1983, previously fixed in late December and early January by the Conseil Supérieur des Corps Universitaires (CSCU), a giant partially-elected parliament of specialist committees. Savary dissolved the previous CSCU, a product of the previous government, a year ago. But this year, without an election policy, each committee has had to be filled by a lottery. It is hoped that the promotions will not be equally random.

Robert Walgate

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French science

No big deal

Eighteen months after the arrival of the Mitterrand government in France, with its promise of a great new deal for French scientists, reform of the Centre National de la Recherche Scientifique (CNRS) is complete, or almost complete.

Last week the new statute for CNRS was finally agreed at the Council of Ministers, and published along with the appointments to seven top jobs — some of them new — and the budget details for 1983, showing continued growth of about 13 per cent in real terms (outside salaries). What remains are the elections to the Comité National, the CNRS "parliament" which makes detailed decisions on grant allocations, appointments and promotions in CNRS laboratories. The elections on new (reformed) principles are planned for March.

M. Maurice Godelier, the anthropologist whose appointment as director of social sciences was vehemently opposed little more than a year ago, has now slipped into the role without much protest. His far-ranging report on social sciences to the minister of research and industry (see *Nature* 28 October, p.771) appears to have had a calming effect, and he has been appointed jointly with the previous acting director, M. Armand Frémont, to a combined directorate of social sciences and humanities. Frémont is expected to concern himself with regional issues, while Godelier will be concerned with the higher issues of policy.

Two new directorates have been created: one for information and publications, and another for the application of research ("valorization"), which should strengthen the role of the CNRS in innovation, a principal objective of the government.

The director of valorization will be M. Jean-Jacques Duby, a 42-year-old mathematician who has had a distinguished career in IBM research and management. M. Duby will consider offering incentives to research groups in the form of additional grants for basic research in return for time spent on applications, a scheme which works well at the Institut Pasteur. CNRS will also seek an increasing role in the research training of third world scientists.

As for the 1983 budget, the net real increase of 7.3 per cent (including salaries), the 320 new research posts (3.4 per cent) and 220 new technicians (1.5 per cent) will be divided unevenly according to the particular needs of each sector. Thus the social sciences receive a large sum for new buildings, to house the home workers; biotechnology sees a start on a new FF20 million (£1.7 million) laboratory for the molecular biology of plants in Strasbourg (which will take three years to complete); and physics sees an allocation for the construction of a new synchrotron radiation source for Orsay, "Super-ACO".

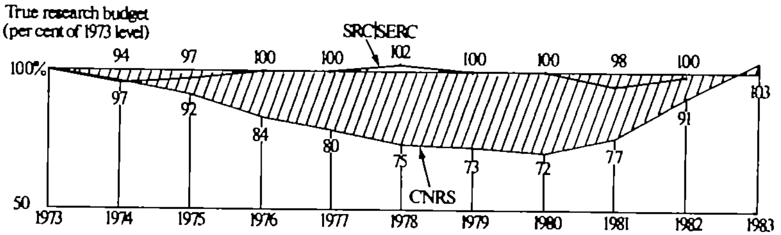
Dividing the budget differently, however, one of the most substantial increases planned for next year comes in medium-sized equipment — instruments costing roughly between FF 1 million and FF 3 million. Here the budget will rise 60 per cent. There is some question, however, whether this sum will survive "regulation" — the mechanism whereby the minister of finance holds back some monies previously promised, subject to good performance of the French economy. This year CNRS did well — losing only FF 50 million in the last analysis, in a budget of FF 6,000 million, but there are fears of worse controls to come — even up to 30 per cent. M. Jean-Pierre Chevènement, the minister for research and industry, is fighting this battle with his ministerial colleagues, in the hope of protecting research from the ravages of the government deficit.

Robert Walgate

Budget trends in Britain and France

For Britain, the graph shows the evolution of the budget of the Science and Engineering Research Council, excepting salaries and corrected for inflation by the retail price index. The figures are given as percentages of the 1973 budget. This indicator of "true money for science" is almost exactly constant over the decade. By contrast, the roughly equivalent Centre National de la Recherche Scientifique in France shows a strong decline in the same quantity over the 1970s, followed by a small rise in 1981 (the last year of the previous government). The strong increase in the past two years indicates the impact of the Mitterrand era. But the 1983 budget will only just pass that of a decade earlier. The continuation of the slope to 1985 is broadly guaranteed by the "law for research" of July this year but may be affected by the uncertain state of the teetering French economy.

Robert Walgate



Professional liability

Biologists at risk

Washington

As biology comes to have commercial implications, biologists need to insure themselves against lawsuits: their professional lives are getting risky. This seems to be the unwritten message of a brochure now being mailed to members of four US scientific societies concerned with biology and biomedicine.

A "professional liability insurance policy" is being offered for $62 per year to members of the Federation of American Societies for Experimental Biology, the American Institute for Biological Sciences, the Society for Neuroscience and the Society for Medical Research. The policy is organized by Maginnis and Associates of Chicago and underwritten by the National Union Fire Insurance Company of Pittsburgh.

The policy covers up to $1 million in costs, in addition to repayment of legal defence costs, for each "occurrence" or incident in which an insured scientist stands accused. It will also pay these costs if the lawsuit arises years after the policy has expired, so long as the events in question took place while the policy was active.

Maginnis officials say the policy covers scientists in situations where they do not normally have insurance cover. An employer such as a university, they note, might be sued for something a faculty member did and then decide the faculty member was negligent and sue him. Or the university might decide that the action in question was outside the faculty member's "scope of employment" and leave him to fight on his own.

The policy would also cover a scientist when consulting outside his regular employment, with a company or individually, whereas his regular employer's insurance would not.

Scientists are covered for advice they give to doctors or nurses about how to treat patients in case the patient later sues the scientist as part of a malpractice suit against his doctors. But the proposed insurance policy does not cover the scientist if he talks to the patient directly, so cancer gene specialists are advised not to hand out free advice to potential patients at cocktail parties.

Arthur C. Gentile, executive director of the American Institute of Biological Sciences, says the policy "covers just about every kind of professional activity". He cites a case where there was an explosion in a laboratory and people who were injured sued. Although the institution that owns the laboratory would probably be liable, the scientist running the laboratory might be named too. "The liability may not be clear and in the meantime you have legal costs" says Gentile.

The policy covers someone charged with libel or slander — such as harming another

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scientist's reputation by overheated statements in the classroom or in published articles.

In the court fight between Hoffman LaRoche and the University of California at Los Angeles (UCLA) over how Hoffman LaRoche obtained a KG1 cell line belonging to the university, the company also named individually two UCLA scientists, David W. Golde and H. Philip Koeffler. It also named Golde separately and accused him of slandering the company. The university is conducting the entire case, including the parts in which the individuals are named, and carrying the costs.

Golde's view is that he therefore does not need the insurance policy now being offered. (Moreover, the university has a malpractice policy that covers him because he is also a physician.) But he warns that "there is not always a 100 per cent congruence of interests between a faculty member and his university in this kind of situation".

As for the likelihood that a university, after defending one of its faculty, would turn round and sue him, Harvard's general counsel Daniel Steiner says it is "far-fetched".

The brochure does not mention the problem of liability for new organisms created through gene-splicing: would the policy cover, for example, a scientist who invents a bug that eats Manhattan, as one observer phrased it? But the policy would in any case be of only marginal use in that situation. For one thing, the likely damages would exceed the $1 million liability limit. Moreover it does not cover any "punitive" damages — the extra penalties a judge can impose to deter the scientist from, say, turning his bug loose on Chicago.

Deborah Shapley

Soviet technology

Red tape blamed for delay

Soviet bureaucrats — not scientists — should be blamed for persistent delays in the introduction of new technology, Academician Anatolii P. Aleksandrov, president of the Soviet Academy of Sciences, said last week. He was commenting on the recent plenum of the Central Committee of the Communist Party of the Soviet Union, which, in his words, had put "new pressure" on the drive for the more rapid implementation of new technology. This drive was a major issue in the policy of the late President Leonid Brezhnev, and the stress that the plenum put on it seems an intensification rather than a change in policy. Dr Aleksandrov, however, may find his own position under the new administration more vulnerable. It is widely believed that, although he was elected by the academy in a secret ballot in the regular way, his candidacy was known to enjoy the support of Mr Brezhnev, and there has been considerable speculation that with Mr Brezhnev gone, Dr Aleksandrov may now be replaced as president of the academy.

In any case, as president of the academy, Dr Aleksandrov holds considerable responsibility for the technological modernization programme, since, during the last five-year plan, the academy was given a major coordinating role and charged with establishing and strengthening links between researchers and industrial customers. This move followed a wave of criticism of scientists for not pulling their weight with those science centres which had been specially created to serve the needs of local industry singled out for special blame — starting with the relatively small Far Eastern Science Centre, and working up to the prestigious Siberian branch of the Academy of Sciences. The scientists retaliated vocally, blaming the administrators and managers who failed to implement their recommendations.

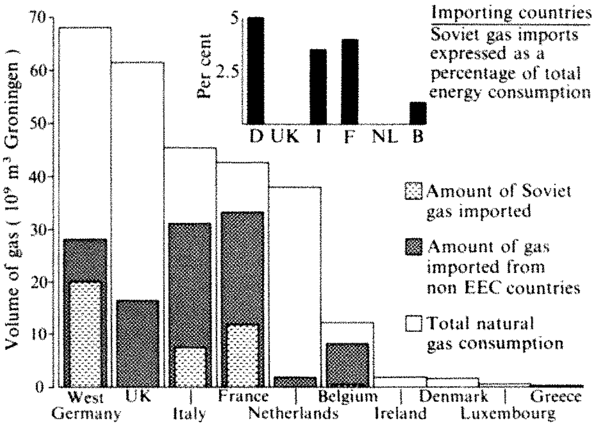
Scientists were again blamed earlier this autumn. On 20 October, *Pravda* carried an article by two senior officials alleging that research potential was being wasted and misapplied in the various institutes of the Academy of Agricultural Sciences. Three days later, in an interview on Moscow radio, Dr Vladimir Shumnyy, deputy director of the Cytology and Genetics Institute of the Siberian branch of the Academy of Sciences, tacitly denied the accusation, citing an impressive list of joint achievements of the Siberian branches of the two academies, from the development of the hardy Novosibirskaya-607 winter wheat and a strain of sheep with a 50 per cent increased wool yield to the hormonal control of plant growth and pig fertility and the protection of grain by irradiation.

Such a media dialogue is a well-known ploy in Soviet journalism; a new drive is launched and backsliders rebuked, and then a selected representative of those under attack states his case. The matter may then be dropped (on the assumption that those who deserved criticism will have profited by the warning), or else may go on to become a full-scale press campaign of criticism, culuminating in a new party and government decree.

What was not routine in this case was, of course, the death of Mr Brezhnev, which necessitated the convening of the Central Committee plenum, and the latter's announcements of, effectively, new party directives. For Dr Aleksandrov, who, only a few days before, had on behalf of Soviet science, made one of the five valedictory speeches at Brezhnev's funeral, it was time to make his position clear. The scientists had produced the designs, he said, and in some cases they were implemented "quite rapidly and effectively". In many ministries, however, the need for updating technology had been discussed for years, but scientists had not yet been able to establish "proper working contacts". The result was waste, inefficiency and delays. The firm reiteration of the problem by the plenum, he hoped, would get things moving again. He stressed, however, that if the scientists' work is not to be wasted, economic managers should be more accountable for their work in implementing new technology.

Vera Rich

EEC dependence on Soviet national gas



The figure shows the dependence of the European Economic Community (EEC) on imports of natural gas with particular reference to the Soviet Union. Natural gas now accounts for 18 per cent of the total energy consumption of the EEC, compared with just 7 per cent in 1970. But fears that demands for imported gas may lead to an increased dependence on Soviet supplies appear to be unfounded, as shown by the situation envisaged for 1990 which is illustrated here. Gas imported from non-EEC countries will account for 44 per cent of the total amount of gas required. Of this, 19 per cent will come from the Soviet Union, accounting for only 4 per cent of the total energy consumption. Other non-EEC suppliers will be Norway (12 per cent of natural gas imports), Algeria (10 per cent) and Libya (1 per cent), and 2 per cent will come from unspecified sources.

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Plant breeding

Genetic diversity threatened

Washington

The International Board for Plant Genetics Resources (IBPGR) is in a race against time to preserve the diversity of germplasm needed for future plant breeding programmes, but its success is threatened by cutbacks in support for international agricultural research.

"If the work is not done in the next 5–10 years, we're finished", Dr J. Trevor Williams, executive secretary of IBPGR, said here last week. The spread of a few high-yielding varieties of many crops "forces the time-scale", and these new varieties are quickly replacing the diverse traditional varieties which have evolved over thousands of years and which represent the essential raw material for the development of future strains. Wild varieties, too, are threatened by such changes as forest clearing in the tropics.

IBPGR, which is part of the Consultative Group on International Agricultural Research (CGIAR), has since 1974 been sponsoring expeditions to gather samples of wild and traditional varieties of key crops and has coordinated efforts to provide storage facilities to preserve these germplasms. But, as a recent report from the CGIAR secretariat notes, "germplasm activity can be discontinued at short notice and, over the short term, its lack is not immediately missed. Over the longer term, its lack will be serious."

CGIAR's support for 1982 is expected to fall about $14 million short of even its low-budget option of $166 million for the year; and given the vulnerability of germplasm activities, IBPGR is "questioning whether it can continue to fund collecting missions or equipment for germplasm storage", according to the report.

IBPGR has sponsored some 250 missions, which have collected about 100,000 new seed varieties. The board has also helped to coordinate the various national germplasm programmes, and has been particularly active in the generally overlooked area of tropical fruits and vegetables. According to Dr William Brown of Pioneer Hi-Bred, a seed company in Des Moines (Brown is also chairman of two crop advisory committees to IBPGR), the board has also played an important part in providing "insurance" in the germplasm resource system. "A real effort has been made to have them stored in more than one place."

The importance of the germplasm collection and storage activities has been underlined by several recent developments. A new variety of rice developed by CGIAR's International Rice Research Institute in the Philippines, for example, included a wild variety of rice (*Oryza nivara*) in its breeding to confer resistance to a disease known as grassy stunt. This new variety, called IR36, has been adopted with remarkable speed, displacing traditional varieties. Almost 11 million hectares have been planted with IR36, which now accounts for more than 60 per cent of the rice grown in the Philippines, Indonesia and southern Vietnam. And, as Dr Williams pointed out, once the traditional varieties are gone, "there is no way you can get them back".

Stephen Budiansky

"Creationism" thwarted

Washington

A Louisiana law requiring the teaching of "creation-science" in public schools was struck down last week in the federal district court. Judge Adrian Duplantier ruled that the law, virtually identical to the Arkansas statute that was overturned last year, infringed upon the authority of the state's Board of Elementary and Secondary Education to set school policy.

In contrast to the Arkansas case, the judge made no finding of fact on the validity of "creationism" as science. "We didn't even deal with the facts", said Burt Neuborne, legal director of the American Civil Liberties Union (ACLU), which brought the suit. "We just said the wrong government agency acted."

Under the Louisiana constitution, the education board has authority to "supervise and control" the public schools; the legislature is charged to "establish and maintain" the schools. The judge ruled that in specifying a detailed course of study, the legislature overstepped its authority. The board, which was originally named as a defendant in the suit, joined with ACLU in arguing that its prerogatives were being usurped by the law. The board, composed of professional educators, is unlikely to institute a "creation-science" teaching requirement on its own. Louisiana's attorney general, however, immediately announced that the state would appeal the decision to the federal circuit court of appeals.

Neuborne suggested that similar separation-of-powers arguments could be raised under the constitutions of other states that pass "creation-science" laws. Georgia and Alabama may do so next year. "Separation of powers is an incredible mine of judicial resources", Neuborne said — and may also be an inexpensive one to tap. "The Arkansas case cost us $1.2 million", he said. "This one cost us eight sheets of yellow legal paper."

Stephen Budiansky

Biotechnology

More help

More help for British biotechnology is on the way. Last week, Mr Patrick Jenkin, Secretary of State for Industry, announced that his department will spend £16 million in the next three years to promote innovation and awareness in biotechnology among British companies. The scheme, modelled on an earlier scheme that promoted microprocessor technology, should go some way to answering persistent criticism of the British government for failing to provide effective support in this area.

In July, a House of Commons select committee complained that the government had failed to protect university research in biotechnology against the reduction of support from the University Grants Committee (UGC) in 1981. Although UGC had set aside £1.2 million for selected universities to support research in biotechnology, the select committee feared that much of this would be used to make good deficiencies incurred in earlier cuts. (The final allocation of this sum has yet to be announced.)

The new scheme from the Department of Industry is intended to complement UGC and research council support, and will be coordinated by a new biotechnology unit set up at the Laboratory of the Government Chemist. A graded system of grants will help companies to commission strategic studies, feasibility studies and specific problem-solving studies by independent consultants. There will also be cash grants for innovative demonstration plants as well as for research projects funded jointly with industry at selected centres. The department also promises a "general strengthening of the biotechnology infrastructure", which will include support for an information service and culture collections.

Mr Jenkin explained that the government was introducing the measures because potentially commercial biotechnology investments entailed high risk and long lead-times. Another reason may be growing concern that British scientists, frustrated by lack of opportunities for research, are being lured abroad. Other recent publicly supported initiatives include a biotechnology research centre at the University of Leicester, supported jointly by the Science and Engineering Research Council and several industrial companies.

Mr Jenkin maintained that British support for biotechnology now compares favourably with that in other countries, an assessment that has been challenged by opposition Labour MPs: one described the scheme as "no more than a drop in the ocean". The Confederation of British Industry has cautiously welcomed the new plan, although a spokesman said of the £16 million figure, "obviously we would like to see it higher".

Tim Beardsley

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Drugs for developing countries

Uncharitable stance from Oxfam

In the poorer countries of the world, millions of people suffer unnecessary ill health or die because simple drugs such as antibiotics are not available. But to what extent have the marketing methods of the multinational pharmaceutical manufacturers contributed towards the misuse and waste of drugs in the Third World?

According to the British charity Oxfam, the manufacturers have blatantly promoted inappropriate and harmful drugs in developing countries, which lack the tight governmental control on drug sale and distribution now normal in wealthy nations. And last week Oxfam renewed its attack on the industry by publishing a new study, "Bitter Pills" by Dianna Melrose. Despite a rider from the charity's director-general Brian Walker, recognizing the "valuable contribution of the research-based drug industry to world health", the study casts the industry as the villain of the piece.

But the industry, in the shape of the Association of the British Pharmaceutical Industry (ABPI), says that until it proves possible to build up adequate health-care systems in the poorer countries so that appropriate drugs can be properly prescribed and distributed, there is little the industry can do.

Abuses cited by Oxfam fieldworkers include: the maintenance of "high prices" for pharmaceuticals; inaccurate or misleading promotional literature; and aggressive promotion of "non-essential" drugs at the expense of more vital products. A common complaint is that over-prescription is encouraged — so, for instance, an impoverished patient with a bacterial infection might be prescribed a tonic and a multivitamin preparation in addition to the vital antibiotic. Since the antibiotic is likely to be the most expensive of the three, and the patient cannot afford them all, the cheaper, heavily promoted tonic and multivitamins may be preferred.

One of Oxfam's answers to over-prescription is to limit the number of drugs on sale in developing countries. The World Health Organization (WHO) has issued a list of approximately 200 "essential" drugs (many others being deemed "useful") for developing countries, and in June Bangladesh's military government took the dramatic step of banning the sale of more than 1,700 drugs termed "harmful" or "unnecessary" and allowing only 150 "essential" drugs from the WHO list to remain on the market (*Nature* 15 July, p.219). Local and international drug manufacturers objected, but although there have been some additions to the list of permitted drugs after strong protests, the government is to go ahead with its plan.

"IMPOSSIBLE – IT SAYS TAKE THREE TIMES A DAY BEFORE MEALS."
BIRCH

Thus events in Bangladesh over the next few years should show just what can be achieved by severely restricting drug supply in a country where many of the problems of distribution, prescribing and poverty persist.

There seems little doubt that a policy based on greater promotion of WHO's 200 "essential" drugs for the developing world will be of some benefit. About 50 drug companies are already cooperating with WHO by promising favourable prices for listed drugs, although further moves like that in Bangladesh actually to prohibit the sale of products not listed would not be likely to encourage further cooperation.

Oxfam, though, seems likely to remain unconvinced. WHO is seen as well intentioned, but "a toothless tiger", in the words of Oxfam's Dianna Melrose, and there is mutual distrust between Oxfam and the drugs industry. Were the two camps to stress the many views they hold in common rather than relish the differences, the world's developing countries could only benefit.

Charles Wenz

Distribution problems — penicillin exposed to heat and sunlight for sale in a market in Upper Volta.

British universities

Agony at Aston

The legal quirk that provides security of tenure for full-time academics at British universities is likely to be tested in a forthcoming battle between the University of Aston in Birmingham and the Association of University Teachers (AUT). The outcome will set an important precedent for other universities.

Aston was told in July 1981 by the University Grants Committee (UGC) that its budget would be reduced by 30 per cent in the succeeding three years. A large-scale programme of voluntary redundancies has already been approved, but now the university's council has voted by a narrow margin (19 votes to 17, with one absentee and one abstention) to effect compulsory redundancies under the 1975 Employment Protection Act. It is thought that 41 academics (chiefly in the fields of engineering and mathematics) could be given notice before Christmas.

The union, according to its general secretary, Mr Laurie Sapper, is prepared to fight all the way. An application to the High Court for a writ to prevent the redundancies is being considered. The grounds of the action are that compulsory redundancies would be contrary to the charter and statutes of the university which (like those of most universities in England and Wales) specify that "no member of staff . . . shall be removed from office . . ." except on grounds such as physical or mental incapacity and immoral, scandalous or criminal behaviour; there is no mention of financial circumstances as a cause for dismissal.

AUT has sought access to the university's accounting figures so that it can carry out its own examination of the university's finances, but Mr Sapper says that Professor Frederick Crawford, Aston's vice-chancellor, has failed to respond to AUT's letters. AUT has accordingly applied for access under the Employment Protection Act.

Aston is primarily technology-based, and has forged extensive links with local industry. The university says that the unemployment rate among its graduates is one of the lowest in Britain.

UGC recommended in July 1981 that student numbers in several science subjects should be reduced and that courses in biological science and architecture should be discontinued. Aston has already axed its courses in human communication and behavioural sciences. The number of science students has fallen from 3,100 in 1980-81 to 2,732 now, while arts student numbers have fallen from 1,383 to 1,340, but further reductions have still to be made. Full-time academic staff, of whom there were 522 in August 1981, will have to be reduced to 357 by July 1984.

At the same time, the university plans to recruit extra staff in some subjects, notably

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for its Management Centre, which received special praise from UGC. It is also determined to maintain a biology presence, possibly by means of a new biotechnology unit. But staff of the existing biology department have refused to examine that plan on the grounds that it would probably involve abandoning the university's established expertise in applied biology and also lead to redundancies.

Dr Richard Etheridge, a senior lecturer in production technology at Aston and a member of the AUT national executive, says that the university staff feel that the vice-chancellor is imposing redundancies with unseemly haste. The Department of Employment was notified of the proposals (as required by employment law) on 18 November, and redundancy notices could be issued as early as 22 December. Several attempts by academic staff at the most recent meeting of the university senate to introduce motions urging reconsideration or delay were blocked by the vice-chancellor on the grounds that these were not procedural matters.

The issue is certain to be raised again at the next meeting of the university council on 14 December. AUT is planning to lobby council members on that day, urging them to reconsider, and has threatened to tell its members elsewhere to boycott the university, depriving it of external examiners and putting existing procedures for the award of degrees in hazard.

Tim Beardsley

Object lesson

Brussels

In an unusual demonstration of its powers, the European Commission has questioned West Germany's second energy research programme, which is suspected of contravening EEC's rules on competition. The West German government has six weeks in which to reply.

The programme in question, which covers the period up to 1985 and costs DM13,465 million (£3,370 million), was in fact approved last year and is already operating. Nevertheless, the Commission complains that it was not informed in due time about either this programme or the last one and points out that the programme is a form of state aid and so is subject to the Commission's approval.

In its letter, the Commission declares that it wants to examine in greater detail the extent to which the programme fits in with the Community's energy policy aims. The fact that much of the money will be set aside for large-scale projects, particularly in fields such as fast-breeder technology, largely carried out in Germany by private companies, leads the Commission to question whether or not the aid is leading to a distortion of competition within the Community. And second, the Commission is concerned about whether only German companies and not, say, the foreign subsidiaries of companies from other parts of EEC, will be able to take advantage of the loans and subsidies.

This is the first time that the Commission has raised objections to a national research programme and its action is being interpreted in Brussels as a sign that research programmes should not be used by national governments as a discreet form of industrial featherbedding. If the Commission is not satisfied with the reply from Bonn, and is successful in pursuing its action, a similar letter might soon be on its way to Paris.

Jasper Becker

Spina bifida

Controversy over tests continues

Heart-searching and a little politicking about the UK Medical Research Council's proposed controlled trial of the utility of folic acid and other vitamin supplements as a prohylactic of spina bifida continues, fanned by the appearance of a blank space in last week's edition (see *Nature* 25 November, pp.302 and 310). What follows is an account of the origin and the course of this controversy.

The immediate stimulus of present interest in a controlled study of folic acid supplementation appears to have been the publication in February 1980 (*Lancet* i, 339) by Professor R.W. Smithells of the University of Leeds and seven collaborators of an article entitled "Possible prevention of neural-tube defects by preconceptional vitamin supplementation". That reported on a study in which women who had previously given birth to a spina bifida child were offered a vitamin preparation containing folic acid. This report appears to have prompted the UK Department of Health to enquire, in 1980, of the Medical Research Council whether a controlled study of the efficacy of folic acid would be feasible and desirable.

Spina bifida results from the incomplete closure of the neural tube in the early stages of embryonic development, and is thought to date from 15 to 20 days after conception. The other principal neural tube defect in human beings is anencephalus. Animal models for the study of neural tube defects, among which the curly tailed mouse stands out, have not so far provided a clear picture of the mechanism by which the neural tube is closed in normal development, although they do clearly show an interaction between genetic and environmental factors in the causation of neural tube defects (see Morriss, G. *Nature* **284**, 121; 1980).

That a dietary deficiency of some kind may be involved in the occurrence of neural tube defects in human births is suggested by the correlation between the incidence of the defects and the social class of the mothers concerned. (The incidence is greater among less prosperous families.) Another pointer of the same kind is the increased incidence of defective births among children conceived in the late winter and early spring.

The geographical variation of the incidence of these defects is, however, harder to account for. In the British Isles, the incidence of both defects is greatest in the west (Ulster and the Republic of Ireland), amounting to 9 per 1,000 in Belfast, 7 per 1,000 in South Wales, 4 per 1,000 in Birmingham (central England) and 2 per 1,000 in south-east England. By contrast, in France the incidence of the defects is less than 1 per 1,000, while the defects are almost unknown among some of the poorest populations of the world. In North America, the incidence of the defects appears to decrease from a maximum on the east coast of about 2 per 1,000 to less than half as much on the west coast. There appears to be a puzzling cyclical variation of incidence in the United Kingdom with a periodicity of twenty years or so.

That genetic factors influence the occurrence of neural tube defects is borne out, for example, by studies in South Wales in the 1960s. In one survey (Carter, C.O. *et al. J. med Genet.* 5, 81; 1968), 5.2 per cent of the siblings of an affected mother were found to produce neural tube defective children, compared with the general incidence of 0.77 per cent in the population as a whole. The risk of neural tube malformations is, puzzlingly, greater among first births and twice as great among females as among male children. One epidemiologist said this week that there can be no other congenital malformation whose incidence provide so many clues but whose causation is still in doubt.

The notion that a deficiency of folic acid may be partly responsible appears to stem from measurements by Smithells and his colleagues suggesting that red-cell folic acid and leukocyte ascorbic acid (vitamin C) might be deficient in mothers giving birth to defective children. These measurements led to Smithells's clinical trial of the efficacy of vitamin supplements including folic acid in the prevention of recurrences of neural-tube defective births.

Women who had previously given birth to spina bifida children were recruited to the study principally from genetic counselling clinics, but also by referral from general practitioners, and were offered a course of treatment with a multivitamin preparation containing folic acid. Among those who accepted, 137 gave birth within the period of the study, as did 187 of those who were found already to have become pregnant again (or who declined). In the first report of the study, among 140 children born to the mothers supplemented with folic acid, there was only one neural tube defect. But there were

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12 neural tube defects among the 192 infants born to the mothers in the untreated group.

More recently, Smithells and his colleagues have extended their study to include more than 1,000 women with a history of neural-tube defective births, self-selected into two roughly equal groups. They report that the incidence of a second neural-tube defective birth was 0.6 per cent (based on three births) in the group of supplemented mothers, but 4.7 per cent among the children born to untreated mothers.

This seven-fold difference apart, the bearing of the Smithells study on the question of whether vitamins including folic acid were an effective prophylactic of the recurrence of neural tube defects is disputed. First, and most seriously, because the study group is self-selected, the results may be seriously biased, and in the direction reported. For if mothers of higher social class are less likely to decline a course of vitamin supplementation, and are inherently less at risk of producing neural-tube defective children, the results of the trial may partially be explained.

On this point, Smithells argues that the data now accumulated include 44 women with more than one neural-tube defective child who have agreed to accept vitamin supplements and 52 women with two or more defective children who had not joined the trial. (The first group produced one defective child, the second five.) To Smithells, however, the significance of this development is to give the lie to the criticism of his earlier work that mothers accepting vitamin supplementation select themselves in consonance with their tendency to produce neural-tube defective children.

Another difficulty is that the catchment area from which the participants in this study have been drawn includes Ulster, where the natural incidence is large, and the south-east of England, where it is small. Recruitment to the programme of vitamin supplementation seems indeed to have been more successful in the south-east of England (50 per cent) than in Ulster (25 per cent).

It seems also to be agreed that the Smithells trial does not point unambiguously to folic acid rather than some other components of the vitamin preparation used as the putatively effective prophylactic. The preparation used (obtainable in the United Kingdom only on prescription) is manufactured by Beecham Pharmaceuticals Limited, is known as "Pregnavite Forte F" and contains a mixture of vitamins (including ascorbic acid) as well as iron and calcium salts.

During the past year, the Medico-Social Research Board in the Republic of Ireland has mounted a trial initially intended to throw light on this point. With the collaboration of several Dublin maternity hospitals, the board is sponsoring a trial in which three groups of mothers with a history of neural-tube defective children are being offered Pregnavite Forte F, Pregnavite Forte (without folic acid) and folic acid alone. Dr Geoffrey Dean, the board's director, said on the telephone last week that "everybody is convinced that the Smithells theory is not proven". The Dublin study also suffers from the defect that there is no objectively chosen control group with which the effects of vitamin supplementation can be compared.

In Dublin there is some resentment that in the past few months Beechams (which had been supplying the vitamin preparations free of charge) has written to say that it cannot continue this provision (see box). Dr Dean hopes that, nevertheless, it should be possible to recruit enough mothers with a history of neural-tube defective children for a significant result to be available within two years.

In Britain, the interest of the Department of Health in 1980 prompted a seminar in December of that year at which several interested and disinterested parties discussed the conduct of a clinical trial intended to yield significant and decisive results, in which the participants would again be mothers with a history of neural-tube defective births.

Beecham's supplement

The decision of Beecham Pharmaceuticals Ltd to go back on its earlier agreement to supply "Pregnavite Forte F" for the Irish trial free of charge, which has caused some ill-feeling in Dublin, is said to have been prompted by the company's internal ethical committee. According to Mr J. B. Diamond, chairman of Beecham (UK) Ltd, the ethical committee includes "secretaries and people like that".

Mr Diamond explained that his company had supplied material for the trials carried out by Professor R.W. Smithells and his associates over a period of some years, and also for the Irish trials. But "when it became clear that the [proposed] trials raised great ethical problems", the committee had decided that it would be wrong for Beecham to supply a placebo for the MRC trial and, by extension, to supply a vitamin supplement containing folic acid for other trials.

On the question of the safety of folic acid as a dietary supplement, Mr Diamond said on the telephone this week that the company had been marketing Pregnavite Forte F since 1952, before the licensing of drugs became the statutory responsibility of the Department of Health on the advice of the Committee on the Safety of Medicines. As such, the product has a licence "as of right", which may nevertheless be called in question whenever the committee considers that there has been a significant change of usage.

The most controversial feature of this proposal is that there should be a true control group — a group of women selected double-blind to whom only a placebo should be administered. Three other groups would receive respectively a multivitamin preparation, a multivitamin preparation with folic acid, and folic acid without other vitamins (but with minerals). The folic acid dose would be 4 mg a day, roughly ten times the amount of folic acid in the Beechams preparation.

The Medical Research Council, which apparently has indications that 20 per cent of the 3,000 women it would need in a full trail are willing to take part, has run into the following snags.

- Several Members of Parliament have been asking questions of the Department of Health about the conduct of the trials. They include Mr Roger Thomas, Mrs Gwyneth Dunwoodie and Mr Leo Abse. The common thread in their questioning has been to ask why their constituents should be denied an apparently effective treatment.
- On some legal views, informed consent by participants in the trial would not necessarily absolve the physicians concerned from legal action of the birth of damaged children.
- The amounts of folic acid planned for the MRC trials (ten times the amounts included in Pregnavite Forte F) have suggested to some that the procedure could be dangerous. While there is no evidence one way or the other, prudence dictates caution.
- If trials should show that folic acid, alone or in conjunction with other vitamins, should be effective at preventing recurrences of neural-tube defective births, it is by no means clear how that knowledge would be used for the prevention of defective first births.

In the circumstances, the opinion about the proposed trials of scientists working in the field understandably ranges over the whole spectrum. Some hold that the Smithells case, if not fully proven, is sufficiently convincing to make the use of a placebo in the trials unethical. Others would avoid the uncertainties occasioned by the choice of a dose of 4 mg a day by arguing that the amounts used should be smaller until animal tests have been carried out.

On the other hand, there are many who hold that the Smithells investigations raise so many more questions than they answer that a trial with placebo is probably necessary. The ideal would obviously be to ring the changes on all the vitamins administered, but this is probably unpracticable in the short term, which is why some argue for detailed physiological investigations of smaller numbers of women at risk.

Professor R.W. Smithells himself hopes that the Medical Research Council "will decide to carry out a trial" at its next meeting on 7 December. ●

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CORRESPONDENCE

Human cancer genes

SIR — In one respect, your News and Views article was startling (*Nature* 11 November, p.103). You have turned upside down the relative values I attach to the different branches of cancer research. You attribute to me the belief that "the prime objective should be to understand the phenomenon of malignancy . . . (rather than to) concentrate on identifying the causes of cancer". This is almost the exact opposite of what I think. In the earlier paper of mine that you cite as evidence of my beliefs (*Nature* **289**, 353-357; 1981), I said that although we know the causes of cancer of the skin and lung, "the preventable causes of the other common cancers are less clear-cut and it may be difficult to identify them until much more is known about mechanism". I go on to say that "eventually the techniques of nucleic acid chemistry should allow us to itemize all the differences in nucleotide sequence and gene expression that distinguish a cancer cell from its normal counterpart, and perhaps at that point the steps involved in carcinogenesis will cease to be in doubt". That was written in 1980, and it still seems a reasonable forecast. Indeed, Logan and I finished the News and Views article that immediately follows your piece with the sentence "But we can at least feel that the technology for studying genes and their patterns of expression are now advancing so fast that the molecular biologist will soon be telling the epidemiologist what to look for".

At the risk of being tediously repetitive, let me say it once again. I believe that the only promising strategy, in the foreseeable future, is to learn what are the causes of cancer and then learn how to avoid them. For, in the absence of prevention, all we are left with is treatment — and that seems, at present, to be an intractable problem.

JOHN CAIRNS

Harvard School of Public Health,
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Cohen–Boyer patent

SIR — In reading the article about Stanford's response on the "second Cohen–Boyer patent" to the US patent office (*Nature* 11 November, p.95), one is struck by the editorializing by the author. The matters brought up by the patent examiner were answered very thoroughly by the patent attorney, and interested readers may obtain the response from the patent office to make their own judgements.

We do, however, wish to respond to the issue raised concerning the possible co-inventorship of Dr Robert Helling, as the article makes it appear the universities have deliberately sought to exclude Dr Helling from being a co-inventor. The facts are these.

Dr Helling has refused to provide evidence supporting his assertion in the press that he is a co-inventor or otherwise communicate with the patent attorney, Mr Rowland, whose professional responsibility it is to determine that the true inventors are indicated on any patent application he files. Under the rules of the patent office, Mr Rowland now is unable to change his original determination of inventorship unless Dr Helling provides the requested information. Issuance of the patent is not affected by this matter of inventorship. If Dr Helling is an inventor, the universities want his name on the patent. Patent office procedures allow for filing of a reissue of a patent to change named inventors.

Lastly, we are disappointed that documents submitted to the patent office regarding inventorship and available to the author of the article were ignored. These would seem to contradict directly statements to *Nature* about Dr Helling's role in the invention.

NIELS J. REIMERS
(Director)

Technology Licensing,
Stanford University,
Stanford, California, USA

Stephen Budiansky responds: The documents Mr Reimers refers to are a short summary by Dr Helling of his research activities, filed with the University of California when he left there in 1973, and submitted to the patent office last month by Mr Rowland. I omitted mention of it because it does not bear on the issue of inventorship. In it Helling states that he "participated in developing a procedure for coupling DNA fragments from any source to bacterial plasmids"; this neither supports nor contradicts his claim that he made a significant contribution to the original conception.

Mr Reimer's assertion that Dr Helling refused to provide information to Stanford's attorney is, I understand, a matter of dispute between the parties, and is in any case academic now that the issue of inventorship is under discussion between Dr Helling, Stanford and the patent office.

Green ecology

SIR — I doubt that I shall be able to dispel the impression gained from the disparaging article "No future for the Greens" (*Nature* 28 October, p.766) but I would like to correct some factual inaccuracies.

The Ecology party conference at Bridlington was not its first public meeting; it has held annual conferences since 1974 and additional policy conferences in 1981 and 1982. All these conferences have been open to outside observers.

Your reporter has also made an error common to ill-informed commentators in suggesting that the Ecology party is solely concerned with environmental issues. As described in its manifesto the Ecology party has policies on all those aspects of life that concern the other British political parties. The difference lies in the application of ecological principles to these concerns in much the same way as the Labour party, for example, would claim to apply socialist principles.

It is correct to suggest that the German Green party (*Die Grünen*) emphasized environmental issues in the recent elections but this does not imply that such issues are the sole concern of the Ecology party. That the Ecology party does not only consider environmental issues may partially explain the greater impact of *Die Grünen*, but the difference between British and German electoral systems is probably more important. The third factor hindering recognition of the Ecology party is the inaccurate reporting in the British media of which the abovementioned article is an example.

Southport, UK R.W. SMALL

Science now and then

SIR — In the course of reviewing Sir Peter Medawar's "Pluto's Republic" (ref.1), Stuart Sutherland commented favourably on the work of K. Popper but less favourably on the work of P. Feyerabend.

Despite Feyerabend's currently anti-Popperian stance Popper[2] has recently drawn a distinction between yesterday's "great science" and today's "big science", indicating that the latter should be attacked along Feyerabendian lines. Popper[3] concluded: "But new ideas should be regarded as precious, and should be carefully nursed; especially if they seem to be a bit wild."

For some of us who were disenchanted[4,5] with the "big science" of the previous decade, Feyerabend was *the* philosopher of science. However, there is evidence that "big chemistry" (at least) is becoming more rational and more tolerant of low status authors[6,7] who publish critical reviews. Thus, Popper's position, strengthened by the recent publication[8] of "Postscript", is becoming increasingly more attractive.

It is currently considered[9-11] that the "chemical revolution" initiated by Lavoisier originated from a Feyerabendian assault rather than from a Popperian "problem situation" or a Kuhnian "crisis". Similar judgements[12,13] are also accorded on Liebig's "biochemical revolution". In both cases the innovators relied on a mixture of empirical evidence and unsupported speculation to justify their theories.

Fruton[14] concludes: "Although most of Liebig's physiological speculations were rejected by several contemporary critics and soon became untenable, there can be little doubt that they stimulated the study of physiological processes from a chemical point of view. The public position that he had won through his achievements in organic chemistry, both as an investigator and as a teacher, assured a respectful hearing for his bold hypothesis. In seeking to prove or disprove Liebig's ideas, his defenders and critics were spurred to examine more closely substances (such as the proteins) present in living organisms . . ."

F.M. AKEROYD

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NEWS AND VIEWS

New lease of life for pulsars?

The discovery of the fastest pulsating radio star so far has surprised observers, and may yet catch out the theorists.

Pulsating stars are becoming fashionable again. That at least is the import of a report in the *New York Times* on Saturday last week, recording the discovery that a well-known but always puzzling radio source, known as 4C21.53, contains as part of its structure a rotating object of some kind spinning at a remarkable 642 times a second. In the past few weeks, most radiotelescopes have been pointed towards the position of the object at Right Ascension 19h 37m 28.72s, Declination 21d 28m 01.3s.

The discovery, made with the help of the radiotelescope strung with wires across the extinct volcanic caldera at Arecibo in Puerto Rico, is credited by the *New York Times* to Dr Carl Heiles of the University of California at Berkeley. The discovery is obviously a landmark in astrophysics because the pulsations of the radio signals are at least twenty times more rapid than those of the most rapidly pulsating star known so far — the supernova remnant more or less at the centre of the Crab nebula. The *New York Times* rightly says that such an object would naturally be taken for a newly formed pulsating star, in which case it might be thought that the radio signals would be accompanied by optical evidence of an expanding shell of matter of the kind supposedly flung off in a supernova explosion and thus travelling through interstellar space at 10,000 km a second or thereabouts.

On the conventional view of the formation of neutron stars as the condensed remnants of roughly one solar mass left over in the explosion of a star perhaps seven or eight times as massive, the rate of rotation of the new pulsating star should also rapidly decay through the conversion of rotational energy into magnetic flux and radiation. The *New York Times* reports that the failure so far to measure such a deceleration is a sign that the pulsating star may not be a now conventional rotating neutron star, but it is probably too soon to jump to that conclusion. The observation of such an object is not child's play, while this particular one has been recognized as a pulsating radio source only since the first half of November (when astronomical observers were informed through the international telegram service) although, with hindsight, it is clear that evidence of pulsation was first obtained during observations in September. The first full account of this discovery, together with a report on what is visible in that patch of sky and a first attempt at an interpretation of these observations will appear in *Nature* before Christmas. On the face of things, the circumstances are even odder than the *New York Times* suggests.

To anticipate what the authors of these discoveries have to say would be unfair to everybody concerned, readers not less than contributors, but just over a decade after the first discoveries of pulsating stars, it is clear that the study of these surprising objects is about to spring to life, and for several different reasons. Not least of all, the mere measurement of a pulsation rate of 642 Hz can be interpreted as the signal from a spinning neutron star only if it is supposed that the object is hovering on the edge of mechanical stability. If such a star has a mass roughly equal to that of the Sun, and a diameter of say 10 km as would be suggested by the equation of state for neutron matter, the velocity of the surface of the star would be a substantial fraction, say 10 per cent, of the speed of light, the rotational energy would be comparable with the energy supposed to be released in supernova explosions and the balance between gravitational attraction and rotational instability would be tenuous, to say the least. It is too soon to know whether some other model than that of the rotating solar-mass neutron star will be necessary to account for the observations now reported.

The other outstanding puzzle about the star itself is the mechanism of its formation. The newly discovered pulsating star, thought to be at a distance of 2500 parsecs, has been radiating for at least twenty years and probably for much longer. It will be crucial to make accurate measurements of the rate of deceleration (reported by the *New York Times* to be negligible). Evidence for radiation elsewhere in the electromagnetic spectrum should also suggest what kinds of processes may have conspired towards the formation of this object (but it is reported that X-ray observations by the Einstein observatory have drawn a blank, which suggests the absence of a recently formed supernova shell). The possibility that the object may have been formed by some process not now considered feasible cannot be discounted, but as yet there is nothing to suggest what that might be.

Puzzles such as these apart, pulsating stars are plainly destined for closer attention because they have proved to be valuable pointers to the characteristics of the galactic environment. So much can be told from the careful measurement of the velocities of 26 pulsating stars measured by A.G. Lyne, B. Anderson and N.J. Salter with the radio-interferometer formed by the antennae at Jodrell Bank and at Defford, 127 kilometres to the south. The sample of radio objects includes those known before 1977, accessible from the Northern Hemisphere and such that a stable reference radio-source could was placed within the working aperture of the interferometer. (See *Mon. Not. Roy. Astron. Soc.*, November 1982.)

The results are important for being the most accurate measurement of the proper motions of pulsating stars, if otherwise unremarkable. So far as can be told from the two-dimensional projection of the velocity vectors on the sky, the motion of this sample of pulsating stars is isotropic. The distribution of speeds differs from a Maxwellian distribution in that there is an excess of high and low speeds — but then there is no reason why pulsating stars at their formation should acquire speeds characteristic of thermal equilibrium. A handful of the objects in the sample have speeds perpendicular to the plane of the galaxy that would be sufficient to carry them beyond the galaxy, while most of the others are destined to oscillate above and below the plane with periods measured in tens or even hundreds of millions of years.

The value of pulsating stars as guides to what the interstellar medium consists of seems also to be destined for fresh appraisal. From the earliest days the rotation of the plane of polarization from a distant radio source (the Faraday effect) has been recognised as a guide to average density of free electrons along the line of sight. Pulsating sources, invariably polarized, are especially convenient sources for this purpose. Now J.A. Roberts and J.G. Ables of the Radiophysics Division of the Australian Commonwealth Scientific and Industrial Research Organisation have formally published (*Mon. Not. Roy. Astron. Soc.*, **201**, 1119-38; 1982) their previously circulated account of how simultaneous records at several frequencies of the variation of the intensity of pulsating stars with time can throw light on the ways in which the radio-energy is scattered in the interplanetary medium. The technique is merely an elaboration of the scintillation measurement that are now customary, but the analysis suggests that it should be possible by a systematic effort to use pulsating stars as a means of keeping track of the gross features of the interstellar radio-scattering material. The only obvious snag is the patience that will be required — and the danger that observers will be dragged away from tasks like these by the discoveries that will succeed the 642 Hz pulsating star.

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Self-splicing RNA

from John Abelson

THOMAS CECH and his colleagues in Boulder, Colorado have been studying the transcription and processing of the ribosomal RNA in the protozoan *Tetrahymena thermophila* and have made a surprising and important discovery — the RNA appears to be able to catalyse the splicing out of its own intervening sequence.

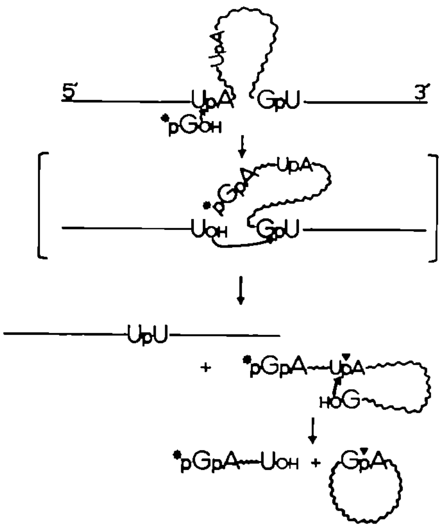
In this species of *Tetrahymena* all of the macronuclear rRNA genes contain an 0.4 kilobase (kb) intervening sequence in the 26S rRNA coding region. Cech found that the excision of the intervening sequence from the precursor RNA, the circularization of the intervening sequence and very likely the covalent joining of the exon sequences are reactions which can take place *in vitro* and do not require either energy (in the form of ATP) or a protein[1].

The conclusion that a protein is not required for the splicing reaction has come from studies on RNA obtained from *in vitro* transcription of a cloned fragment of the 26S rRNA gene from *Tetrahymena.* Earlier studies by Cech and colleagues[2] on the splicing of RNA transcribed in isolated *Tetrahymena* nuclei had suggested that the splicing reaction had unusual properties. The excision of the intervening sequence during *in vitro* transcription depends on monovalent cation concentration. In low salt, transcription of the rRNA genes is optimal and no intervening sequence is excised. At higher salt concentrations, the intervening sequence is released as a linear RNA molecule which is subsequently converted to a circle.

Using precursor RNA synthesized in low salt, the requirements for excision and circularization of the intervening sequence were found to be a monavalent cation (NH_4^+), a divalent cation (Mg^{2+}) and guanosine. The requirement for guanosine can be met by GTP, GDP, GMP or guanosine but not by 2′-deoxyguanosine, 2′-3′-dideoxyguanosine or 3′-GMP. Thus, there is a requirement for the 2′- and 3′-hydroxyls of guanosine but the hydrolysis of nucleotide is apparently not required for energy. When the precursor RNA was deproteinized by a variety of rigorous protocols including SDS–phenol extraction, boiling and protease treatment, it remained active in the excision reaction, requiring only salt, Mg^{2+} and guanosine.

If the G in the reaction is labelled, for example as ^{32}P-GTP, the label is found at the 5′ end of the linear intervening sequence but not in the circular intervening sequence. During excision of the intervening sequence, the guanosine cofactor is linked to the intervening sequence in a 3′–5′ phosphodiester bond[3]. The circularization of purified linear intervening sequence requires only added divalent cation and proceeds better at elevated temperature. The products of the circularization reaction are a covalently closed circle that is 15 bases shorter than the linear intervening sequence and a linear fragment containing 15 bases of the 5′ end.

These studies suggested that the ribosomal RNA was capable of self-splicing but the presence of a peptide with unusual properties was difficult to exclude. To deal with this, Cech and his colleagues[1] have now cloned a fragment of the *T. thermophila* 26S rRNA gene into a plasmid containing the *Escherichia coli lac UV5* promoter. *In vitro* transcription of this DNA with purified *E. coli* RNA polymerase produced a transcript containing 35 bases of *lac* sequence, 261 bases of the 5′ exon, the 413 base intervening sequence and 624 bases of the 3′ exon. This transcript was deproteinized and was shown to undergo self-catalysed excision and circularization of the intervening sequence. In addition, the products of splicing were investigated by nuclease S_1 mapping using a DNA fragment lacking an intron. The results of the study were compatible with precise exon joining.



The splicing reaction mechanism.

These results require a model that by necessity takes us into unfamiliar territory. We have an RNA that is apparently capable of a series of phosphotransfer reactions. The reactions are self-catalysed. The RNA is not an enzyme since there is no evidence that any RNA molecule can do more than catalyse self reaction. Cech and colleagues suggest the new term 'ribozyme'. Whether the reaction is catalysed by an enzyme *in vivo* or not remains an open question but there is no *a priori* requirement for an enzyme. The excision reaction is only 60 times faster *in vivo* than *in vitro* and this difference can easily be explained by factors present in the cell which might stabilize the RNA structure.

In the present working model of the splicing reaction, which will be described in detail in a paper soon to be published in *Nature*[4], the splicing reaction is initiated by the binding of G to a site in the RNA in close proximity to the 5′ splice junction (see the figure). The phosphodiester bond at the 5′ splice junction is broken, perhaps due to strain provided by the tertiary structure of the RNA, and the 5′ phosphate is transferred to G forming a 3′–5′ phosphodiester bond. The 3′ splice junction is similarly opened with transfer to the 3′ hydroxyl of the 5′ exon to form the spliced rRNA. The linear intervening sequence is released. In the subsequent circularization reaction a phosphodiester bond 15 nucleotides from the 5′ end of the intervening sequence is opened. In this case, transfer is to the 3′ end of the intervening sequence to form the circle with the release of the 5′ oligonucleotide. In this mechanism, each of the three cleavage reactions is accompanied by a ligation reaction, that is, the process consists of a series of three phosphotransfer reactions. These reactions are expected to be reversible. Presumably they are driven in the direction of excision by the high concentration of guanosine and perhaps by changes in the structure of the RNA.

The unfamiliar aspect of this mechanism is, of course, that we must assume that the RNA is folded in such a way as to form a guanosine-binding site and to bring the phosphodiester bonds which are to be exchanged into very close proximity. Water must be excluded from the active site to prevent hydrolysis. There are other examples of phosphotransfer reactions which do not require energy, notably the DNA nicking-closing enzymes.

The mechanism of RNA splicing described here is, by its nature, likely to have been a primitive one. One might expect that all RNA splicing would proceed by a similar mechanism; but this is not the case. In the other system where detailed information is known of the mechanism — tRNA splicing in yeast — the reaction proceeds by a totally different mechanism. At least two enzymes catalysing as many as four reactions are required and the ligation step requires two molecules of ATP[5,6]. Little is known about the mechanisms of mRNA splicing but it will not be surprising if that reaction proceeds by yet a third mechanism.

Since intervening sequences have only been found in the genes of eukaryotes, it has been assumed by some that splicing is a reaction which emerged rather late in evolution. The discovery of self-splicing RNA brings that assumption into question.

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If an RNA molecule can have the intrinsic property of specifically breaking and rejoining its own bonds and of forming specific binding sites for nucleotides, certainly those properties would have been put to use in early evolution. As Cech and his colleagues point out, the reverse of the excision reaction is integration. This raises the possibility of 'RNA transposons'. Shuffling of RNA sequences by these mechanisms could have been an important factor in the generation of sequence diversity in primordial organisms. ☐

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Inserted retroviruses cause embryonic lethal mutation

from Keith R. Willison

EXPERIMENTS in which genes and recombinant DNA molecules have been transferred heritably into the chromosomes of mice have resulted in the identification of a gene whose expression may prove to be essential for a specific stage of embryonic development. Although natural stage-specific embryonic lethal mutants are known, none of the mutant genes has yet been isolated and their mode of action has remained unclear. Rudolf Jaenisch has now used the alternative approach of interfering with the expression of a normal gene through the insertion of retroviral sequence into a mouse chromosome. This has enabled him to produce artificially an embryonic lethal mutation whose site of action he can locate with a retroviral probe.

This experimental approach and its possible applications have been discussed in earlier *News and Views* articles[1,2]. Jaenisch decided, some ten years ago, to try to introduce novel genetic material into the mouse and study its effect on development by infecting early mouse embryos with tumour viruses. Both DNA and RNA tumour viruses were tested and it was quickly found that the Moloney murine leukaemia virus (M-MuLV) was the most promising in the sense that the viruses were able to become integrated in the DNA of the sperm and eggs[4] and could be inherited by later generations of mice. This in itself was an important result at the time (before the advent of gene cloning) because it proved that such events could be made to occur in the laboratory as well in the wild. That this does indeed happen in natural environments has been amply confirmed in cats[5], mice[3,6] and chickens[7]. Some of the mice which carry M-MuLV sequences eventually express them in lymphatic tissues, become full of virus and develop leukaemia.

Jaenisch has constructed 13 mouse strains containing integrated M-MuLV sequences called *Mov 1–13*. They were produced by infecting embryos at different stages with virus or purified DNA using microinjection techniques[4,8,9,10] (see also ref. 1 for more detail). The strains are maintained as heterozygous stocks in order to make it easier to breed healthier animals, and the M-MuLV-containing chromosome is followed either by measuring viraemia in the offspring or by Southern blotting with purified M-MuLV probes. The latter technique allows one to distinguish between animals homozygous or heterozygous for the *Mov* allele in cases where the genomic flanking sequences have been cloned, and therefore aids breeding for *Mov*-homozygous mice.

The construction of homozygous strains was described by Jaenisch at a recent meeting* of the Ciba foundation in London and has given an important result. The attempt to produce *Mov-13* homozygotes resulted in consistently smaller than expected litter sizes for the particular inbred genetic background on which it was carried (C57BL/6). This suggested that some of the embryos might be dying, that is, that the *Mov* allele was behaving as an embryonic lethal gene. In fact it turned out that the homozygous embryos (*Mov-13/Mov-13*) were dying at around day 12 of gestation. There appeared to be no specific malformations but rather a total degeneration of the embryo. As the DNA does not quickly break down in dying embryos, it could be isolated and analysed to show that the degenerating embryos are indeed homozygous for *Mov-13*. The lethality of *Mov-13* homozygotes was stage-specific and absolute; 55 embryos surviving beyond day 14 were analysed and none was homozygous for *Mov-13*.

Are there precedents for the production of mutations by retroviruses? It is worth noting that a natural integration of an

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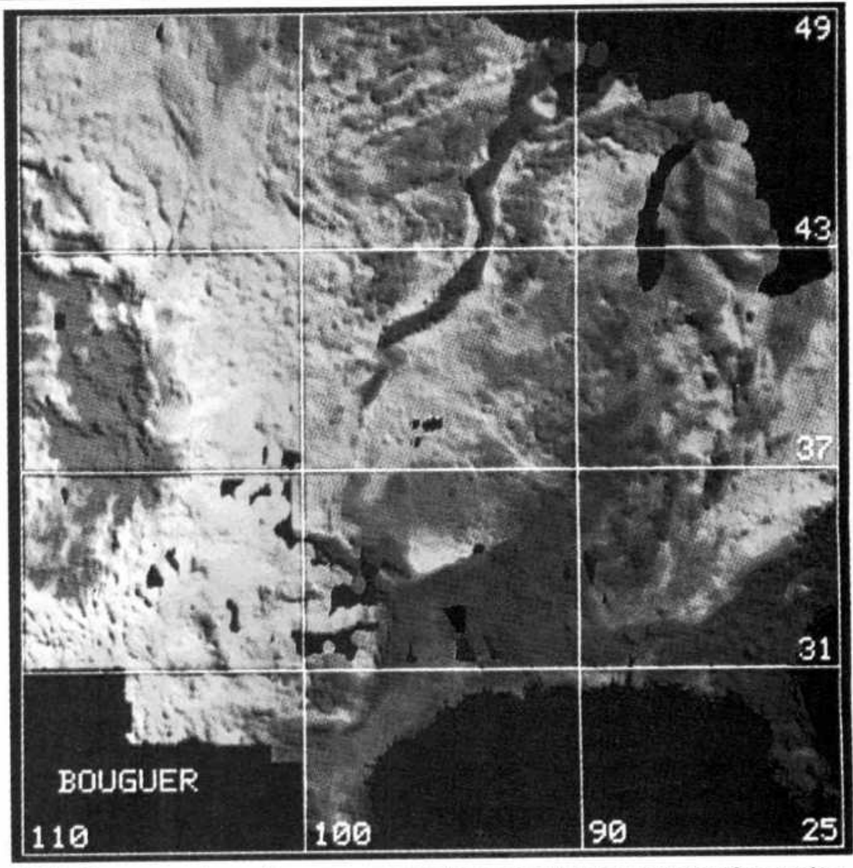
*The Ciba Foundation meeting on the 'Molecular Biology of Oogenesis' was held 9–12 November. The proceedings will be published as Ciba Symposium 98 (Pitman Books).



Digitally processed gravity measurements have revealed the presence of an ancient rift structure extending across Missouri. In this image the rift extends from the top (centre right) to the middle (centre left). The structure, 120 to 160 km wide and 700 km long, is not discernible from surface topography. E.A. Guiness *et al.* (*J. geophys. Res.* **87**, 8529; 1982) identify the feature as the signature of a Precambrian rifting event that failed to develop into a spreading zone.

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MuLV provirus has also given rise to the dilute (*d*) coat colour mutation of DBA/2J mice[11], and in tissue culture Varmus[13] has used M-MuLV as an insertion mutagen to isolate revertants of a rat cell line transformed by Rous sarcoma virus. In this latter case it was directly shown that the M-MuLV had inserted into the resident RSV provirus sequence in the rat genome and affected the expression of RSV RNA species.

How does the *Mov-13* allele cause embryonic death? Is it just toxicity due to viraemia? No virus can be detected in heterozygotes until day 16 of development and viraemia would certainly be expected to be dominant. Has the M-MuLV DNA integrated into a gene or gene complex essential for embryonic development? It has certainly inserted into actively transcribed DNA, as Jaenisch also reported at the meeting. Furthermore, transcription of DNA at this site normally begins at day 12 of gestation when in normal embryos two abundant transcripts of 4kb and 5kb can be detected in the total RNA. The level of expression of these two transcripts changes during development, maximum expression occurring between days 14 and 18 when at least 10-fold more 4kb and 5kb RNAs can be detected than on either day 12 or day 19 of development. There appears to be no expression of these transcripts in adult tissues, although very small quantities of a 7–8kb transcript are detectable. The relationship to the embryonic 4–5kb RNAs is not yet known. A reasonable interpretation of these data is that an embryonic lethal mutation has been produced by insertion mutagenesis. The affected gene appears to be expressed primarily during embryogenesis and its identification and cell distribution will be awaited with great interest. ☐

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Chromatin structure, DNA structure

from Sarah C.R. Elgin

DURING the past few years, specific features of eukaryotic chromatin structure, in particular sites hypersensitive to digestion by DNase I, have been detected and mapped for a variety of genes[1–4]. Frequently (but not always) such sites have been observed at or near the 5′ends of genes transcribed by polymerases I and II (see ref.5 for a brief review). Several studies have noted that 5′DNase I-hypersensitive (DH) sites are present before transcription begins, suggesting they may have some functional role[2,4,6,7]. Now, a number of recent studies is providing evidence that DH sites can play a critical part in the control of gene expression.

A cluster of five DH sites, found only in nuclei where the gene is expressed, has been mapped upstream from the *Drosophila melanogaster* glue protein gene *Sgs4* (ref.8). In a strain that does not express *Sgs4* (ref.9), the DH sites are not observed[8] and sequence data reveal that a small upstream deletion has removed the major DH site of the complex[10] — implying that the loss of this segment both affects the chromatin structure of the surrounding region[8] and eliminates gene expression. A clue to a possible underlying mechanism comes from work on the chick globin locus[11]. Here DNA sequences at or near the previously mapped chromatin DH sites can adopt (at least) two different conformations. When relevant DNA segments are subcloned into pBR322 and the recombinant plasmids are digested with S_1 nuclease, these sites are cleaved in the negatively supercoiled DNA molecule, but not in the linear DNA molecule[11]. We may find that there are switches relevant to gene expression constructed from DNA sequences that can possess alternative structural configurations, the different forms being stabilized and amplified in the chromatin structure. The major DH site of the *Sgs4* locus is a good candidate for such a switch.

The *Sgs4* glue gene of *D. melanogaster* is normally expressed in the salivary gland of the third-instar larva. Differences in mRNA production, observed for different strains, correlate with the presence of small upstream deletions[9,10]. Three Oriental strains (Seto, Hikone-R and Kochi-R) that produce only trace amounts (~2 per cent of levels observed in Oregon-R) of the *Sgs4* mRNA have an upstream restriction fragment that is shorter by about 50 base pairs (bp) than that of five strains (Oregon-R, Chiete V, Urbana-S, Canton-S and D323) that produce significant amounts (~40–100 per cent) of the mRNA. Sequencing of the cloned genes from Hikone-R and Seto flies confirms a deletion (marked ORL on the figure) from –305 to –356 (replaced by the nucleotides CGC) relative to Oregon-R. In the Berkeley-1 (BER-1) strain no *Sgs 4* mRNA has been detected at any larval, pupal or adult stage tested. Here, sequencing of the cloned gene confirms a 95 bp deletion from –392 to –486 (replaced by GAT). Both producer and nonproducer strains have the same TATA box at –22 to –28 and essentially the same loose match with the consensus sequence $5'$-GG$^{C}_{T}$CCAATCT-$3'$ at –66 to –74 (the only change being the substitution of a C for the $3'$-terminal T in BER-1).

In nuclei from embryos or tissue culture cells, three DH sites can be observed $3'$ to the *Sgs4* gene but none near the $5'$ end. The $3'$ sites persist in the chromatin of the third-instar larval salivary gland, but in addition major sites can be mapped to +30, –70, –330, –405 and –480 (with an estimated accuracy of ±30–50 bp) relative to the start of transciption. The most prominent site is that centred at –405 (mapped within ±5 bp). The site mapped to –330, which is lost in the Oriental strains, might play a part in determining the efficiency of transcription. The other DH sites are still apparent in the chromatin of these larvae at the same DNA sequences[8]. In contrast, all the tissue-specific $5'$ DH sites are lost in the nonproducer BER-1 strain[8], even though there are no major differences observed in the DNA sequence other than the deletion[10]. In particular, the sequences from –390 to –250 are identical for Oregon-R and BER-1 flies. This indicates that the DNA sequences at or near the major DH site have a role in determining the chromatin structure of the surrounding region.

That this function may reflect a switch in the underlying DNA structure is suggested by the work on chick globin genes described above. It must be noted, however, that not all sites which are S_1 sensitive in supercoiled plasmids correspond to known DH sites in chromatin[11]. Conversely, it seems likely that not all chromatin DH sites will be S_1 sensitive. Nonetheless, it is apparent that in some instances, specific DNA sequences near the $5'$ ends of globin genes can adopt different conformations dependent on supercoiling of the DNA. That such a switch occurs *in vivo* is indicated by the observation that S_1 nuclease also detects sensitive sites in chromatin, in the region of the previously characterized tissue-specific and developmentally specific DH sites[11]. The same sites are recognized by reagents such as bromoacetaldehyde, which react with unpaired regions of DNA (T. Kohwi and H. Weintraub, personal communication).

What might be the nature of this change? What sequences might be involved? Certainly the most radical suggestion would be

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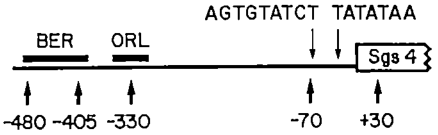

Map of the 5′ end of *Sgs4* from *D. melanogaster* Oregon-R. DH sites are indicated by broad arrows. The positions of the control sequences and deletions discussed are indicated.

a switch in the form of the DNA double helix, for example from B to Z. This transition can occur in physiological conditions in a segment of d(pCpG)n, the Z form being stabilized by the introduction of negative supercoiling[12,13]. It seems likely that Z-DNA could also be stabilized by protein binding[14]. The B–Z boundary is sensitive to S_1 nuclease[13].

However, a less radical shift in DNA structure seems more likely. No common features of sequence organization have so far been identified for DH sites. It has also been reported that enhancer elements of similar function, such as those of SV40 and of polyoma virus, do not show any obvious sequence homology[15]. It is possible that recognition of such sites will come only with a greater appreciation of the three-dimensional structure of the DNA. That the conformation of the DNA double helix, including the structure of the sugar–phosphate backbone, is variable and sequence dependent, even within the overall B form, is clear both from recent studies of the helical periodicity[16,17] of B-DNA and from the X-ray crystallographic analysis of the B-DNA dodecamer CGCGAATTCGCG[18]. The sequence-dependent conformation is apparently reflected in the sensitivity of the DNA to endonucleases, there being a good correlation between the sites of rapid DNase I cutting in this dodecamer and positions of high local helical twist[19]. The rate of cleavage at a particular site can reflect not only the immediate site but also the 'DNA environment', as illustrated by the fact that the four different *Pst*I sites in pSM1 are cut at very different rates[20]. This effect is not dependent on the degree of supercoiling. It seems, then, that it may be possible to obtain empirical maps of DNA structure by examining site-specificity in the interaction of DNA with enzymes (such as micrococcal nuclease)[21] or with various small DNA-binding compounds (for example intercalators)[22,23]. Structural patterns in the DNA that are invariant to supercoiling might be related to relatively stable features of chromatin structure, while those that are altered might be candidates for potential switch regions as discussed above. Clearly a more detailed appreciation of DNA structure is required to understand the code that directs protein-DNA interactions in eukaryotic chromatin. □

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Chromosomes and cancer

from David Forman and Janet Rowley

THE analysis of chromosomes in relation to cancer is a field of research which has gone through successive periods of excitement and frustration. The enthusiasm in the early 1960s arising from the discovery that chronic myeloid leukaemia is strongly associated with a specific marker (the Philadelphia chromosome Ph^1; see also Logan, J. & Cairns, J. *Nature* **300**, 104; 1982) diminished when it was realized that few other cancers exhibit such clear-cut abnormalities. Since then there have been critical developments in technique which have brought the chromosome back to the centre of cancer science.

These technical developments have been of two types. First came consistent improvements in the banding of human chromosomes, which revealed several translocations and deletions, beginning with the discovery that the Ph^1 chromosome results from a translocation usually involving chromosomes 9 and 22. Second, and more recently, has come the application of recombinant DNA technology to the human genome. As was emphasized at a recent meeting on chromosomes and cancer*, the result of these changes has been a narrowing of the gap between molecular biology, dealing with units of around 10^3 nucleotides upwards, and cytogenetics, dealing with around 10^6 nucleotides downwards. Although the gap is not yet closed, communication between the two fields is becoming intense and vigorous. Not only are more cancers being related to consistent non-random chromosome changes but important clues are also being found as to how these alterations affect function.

The most exciting advances reported at the meeting came from Croce (Wistar Institute) and Taub and Leder (Harvard Medical School) concerning Burkitt's lymphoma — already known to be closely associated with an (8;14) translocation. Chromosome 14 contains the immunoglobulin heavy-chain gene cluster which is active in Burkitt's lymphoma cells. Both groups showed that the break in chromosome 14 occurs within the cluster and that the break in chromosome 8 is at the site of the *c-myc* oncogene. Croce and co-workers at the Wistar Institute found the heavy-chain gene regions from several independently derived Burkitt's lymphoma cell lines hybridized with *c-myc* probes. The Harvard group, using a probe derived from a mouse plasmacytoma, isolated a homologous segment of DNA from 12 Burkitt's lymphoma cell lines. In two cases a single restriction fragment contained both a portion of the heavy-chain gene and the cellular *c-myc* gene. Both groups found that about a third of all lines tested contained rearrangements of the immunoglobulin heavy-chain region caused by the integration of the *c-myc* region. Croce suggested that in these cases the genes are aligned in a 5′ to 5′ head-to head fashion. The suggestion is therefore that the level of expression of the transforming *c-myc* gene after translocation is affected by the neighbouring active immunoglobulin genes and normal regulatory control is lost. It is not without significance that the other chromosomes involved with chromosome 8 in translocation events in Burkitt's lymphoma are numbers 2 and 22, containing the sites for the immunoglobulin κ and λ light-chain genes respectively, and that the lymphomas express κ chains in the case of (8;2) translocations and λ in the case of (8;22) translocations.

An animal B-cell tumour, the mouse plasmacytoma, also involves an homologous translocation — a (12;15) or occasionally a (6;15) translocation (Spira, Karolinska Institute). Again chromosome 12 contains the mouse immunoglobulin heavy-chain gene and chromosome 6 the κ light-chain gene. The *c-myc* gene is known to be located on chromosome 15 in mouse

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*The fifth Bristol-Myers symposium on cancer research — 'Chromosomes and Cancer From Molecular to Man' — was held at the University of Chicago on 18–19 October 1982

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and several investigators, as well as the group at Harvard, have shown that it is translocated adjacent to the immunoglobulin locus.

If *c-onc* genes in aberrant positions are critical to tumour development, what is their normal function? Lane and Cooper (Sidney Farber Cancer Institute) have identified five different stage-specific transforming genes active in lymphocytic leukaemias and lymphomas. Tumours which share a common phenotype, representative of a discrete stage in normal lymphoid differentiation, appear also to share a common transforming gene, suggesting that the altered genes may normally be involved in growth regulation during cellular differentiation. The investigators have gone on to examine a series of African and American Burkitt's lymphomas by transfection of NIH 3T3 cells and have identified a common transforming gene activated in these tumours which is not analogous to genes from the Epstein–Barr virus genome or indeed to *c-myc*. Thus different genes may be involved at different stages of the neoplastic process and in the world outside NIH 3T3 cells, tumour development may depend on the activity of more than a single oncogene.

In this respect it is interesting that the oncogene isolated from a bladder carcinoma cell line and which is homologous to the *ras*^{H} gene maps to the exact region of chromosome 11 that is deleted in the Wilms tumour–aniridia syndrome (11 p13; Francke, Yale University). Thus here there is an association between one particular oncogene and two quite different tumours. It is also apparent that particular translocations may be associated with variable but related tumour phenotypes. The (9;22) translocation is involved in certain cases of acute lymphocytic and non-lymphocytic leukaemias as well as in established chronic myeloid leukaemia. High-resolution banding techniques have shown that the break point on chromosome 22 is very similar in all three diseases (Yunis, University of Minnesota). If there were an oncogene associated with either chromosome 9 or chromosome 22, additional factors must determine which type of phenotype is expressed.

In general, these new advances emphasize the contribution made by cytogeneticists in providing an expanding data base which allows molecular biologists to decide which areas of the genome to concentrate on. Yunis and co-workers can now distinguish several thousand chromosome bands in the human genome so that previously masked small chromosome defects can now be visualized. Certainly it is clear that chromosomal abnormalities are the rule rather than the exception in haematological malignant diseases, with over 90 per cent of all types of acute leukaemia exhibiting some form of defect.

A computerized cancer chromosome registry has now been established (Metelman, University of Lund) and is revealing that although most leukaemias are associated with several types of defect, there are distinct patterns in these associations. First, the abnormalities usually only involve a limited number of chromosomes. In the case of acute myeloid leukaemia, although there is not an association with one specific change, there is a strongly preferential involvement of chromosomes 5,7,8,15,17 and 21. Second, it is usually the case that specific segments of the chromosomes are involved in the aberrations (for example, bands q33–36 on chromosome 7). Third, detailed analyses are making possible the beginnings of a cancer chromosome epidemiology. For example, the incidence or frequency of chromosome changes associated with acute non-lymphocytic leukaemia (ANLL) that arise from chemotherapy or irradiation following a previous malignancy are different from the changes that take place when the disease occurs *de novo* or in childhood (Rowley, University of Chicago). In childhood an (8;21) translocation or an 11q deletion is often seen in ANLL. With increasing age and with exposure to chemicals, either occupationally or as a result of cytotoxic therapy, deletions of part or all of chromosomes 5 or 7 become much more prevalent and the (8;21) or 11q effects very rare. The specific type and degree of abnormality can often have clinical implications in terms of both diagnosis and prognosis (Golumb, University of Chicago).

Further technical develpments will soon be ready to be profitably employed in research into chromosome neoplasia. Flow cytometry can now individually separate most human chromosomes and has already been used for mapping studies (Carrano, Lawrence Livermore, National Labs) and the preparations of single chromosome DNA libraries (Young, Beatson Research Institute). Ward (Yale University) described the *in situ* hybridization of as little as 20–30 pg of biotin-labelled DNA to the chromosome with subsequent detection and visualization using the protein avidin and alkaline phosphatase. It should soon be possible to rapidly screen individual chromosomes with probes for specific genes and build up a much more detailed account of the structure of the chromosome in neoplastic cells.

The increasing fund of knowledge at both the cytological and molecular levels, together with the arrival of new techniques, should lead to rapid advances in our understanding of the role of chromosomes in cancer and, hopefully, to much improved therapy. □

Sex reversal and sex determination

from Elizabeth Simpson

THAT mammals having the XX sex chromosome constitution are generally female, while those with XY constitution are generally male has often been interpreted as showing that a gene (or genes) on the Y chromosome controls primary sex determination. When a Y chromosome is present the foetal gonad, which has the potential to become either ovary or testis, differentiates into a testes and, when absent, into an ovary — the first and crucial event in a series of changes that determines whether the organism has a male or female phenotype. Recent genetic analysis shows that this first step is not as simple as first thought and appears to be controlled by an interaction between autosomal genes and a gene(s) on the Y chromosome, for normal male differentiation.

Insight into sex determination has been provided by exceptions to the rule that the presence of the Y chromosome leads to a male phenotype. In mice, which provide the most readily examined examples, sex reversal can be partial or complete, and is characterised by XX individuals that have testes (rarely ovotestes) and are apparently normal phenotypic males, and XY individuals that have ovotestes or ovaries, and are apparently normal or near normal phenotypic females.

The *Sxr* (sex reversed) gene, originally described by Cattanach, causes XX mice inheriting it to develop as phenotypic males. It was at first thought to be a dominant, autosomal gene, probably derived by translocation from the Y-chromosome. However, it proved impossible to map the *Sxr* gene to any of the autosomes — a mystery that has only recently been solved by the finding that *Sxr* is located on the distal arm of the X chromosome in XX sex-reversed mice.

The mapping of the *Sxr* gene on the X chromosome comes from two independent lines of evidence. First, experiments described in this issue of *Nature* by McLaren and Monk (p.446) and Cattanach and colleagues (p.443 & p.445) show that some *Sxr* XX mice develop as females or hermaphrodites rather than males if the

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paternally inherited X chromosome is preferentially inactivated (paternal X inactivation was ensured by introducing Searle's translocation (T[X;16]16H[3]) through the mother). Second, *Sxr* has been mapped to the X chromosome by Singh and Jones[4] using hybridization *in situ* of Y chromosome specific DNA sequences (Bkm) to the distal end of the X chromosome in XX *Sxr* mice. From these findings Eicher[5] and Burgoyne[6] have gone on to propose that the Y chromosome contains two regions of Bkm positive sequences, one in the normal centromeric position and the other at the distal end of the chromosome. This makes it possible to explain why sex reversal is inherited in a pattern characteristic of an autosomal dominant gene, with half the XX progeny being sex reversed and half the XY progeny carrying the *Sxr* gene. During meiosis, when haploid sperm are generated by a carrier male, crossing over between X and Y chromosomes creates four types of sperm (i) an X carrying the translocated Bkm sequences from Y, (ii) a normal X, (iii) Y carrying both Bkm sequences, and (iv) a normal Y; created by the transfer of the distal Bkm sequence bearing end of the carrier Y to the X. Assuming that each sperm type has an equal chance to fertilize ova, this would produce the observed 1:1:1:1 ratio of *Sxr* XX males, XX females, XY *Sxr* carrier males, and XY noncarrier males. This interpretation of the transmission of *Sxr* is supported by recent cytogenetic data (Evans, Burtenshaw & Cattanach, this issue of *Nature*, p.443).

Burgoyne[6] has also argued that there may be a whole class of 'pseudo-autosomally' inherited genes located distal to the crossover point on the pairing segment of the X and Y chromosomes. These genes normally escape X inactivation and like *Sxr* would show the same pattern of apparently autosomal inheritance. Some of these genes, in addition to the normal Y specific Bkm related sequences present in the non-pairing part of the Y chromosome, may be involved in sex determination.

Recent work of Eicher and her colleagues[5] with a series of XY sex reversed mice have indicated that the testis determing gene *Tdy* which is probably closely linked to the Bkm sequences, and maps near the centromere on the Y chromosome, cannot be the only primary sex determining gene: it needs to interact with other genes, perhaps in appropriate sequence for normal sex determination. One set of Eicher's sex reversed mice show inheritance of abnormal sex determination through a Y chromosome derived from *Mus poschiavinus*, a subspecies of *Mus domesticus*. An inbred strain of mice with this Y chromosome, designated Y^{pos}, breeds normally, and produces normal F_1 hybrids when mated with the C57BL/6 laboratory strain. However, when these hybrid males are backcrossed with C57BL/6 females half of the XY progeny are hermaphrodite (one gonad being an ovotestis). In subsequent backcross generations the proportion of abnormal XY individuals increases until a high proportion of them are completely sex reversed, phenotypic females with two ovaries, and the rest were males with ovotestes, most of which are fertile[7].

That there is a 1:1 ratio between males and hermaphrodites among the XY progeny of the first backcross generation argues for an effect of a dominant autosomal or pseudoautosomal gene (named *Tda-1*[5]) in addition to the abnormal testis determining gene *Tdy* transmitted by Y^{pos}. The increasing incidence of complete sex reversal in XY individuals in sequential backcross generations argues for the involvement of additional autosomal gene(s). Thus it would appear that in the original Y^{pos} carrying strain, the testis determining gene, *Tdy*, on Y^{pos} could interact normally with a dominant autosomal gene, *Tda-1*, and with additional autosomal gene(s) perhaps in carefully timed sequence, to determine primary sex (testis or ovary). However, when *Tdy* POS was isolated by backcrossing onto the C57BL/6 inbred genome, it could not interact normally with the corresponding genes of C57BL/6.

Three other examples of the type of sex reversal seen with Y^{pos}, have now been found in wild mice captured in Northern, Central and Southern Italy respectively. Each is inherited via the Y chromosome of a male *Mus domesticus*, and produces XY hermaphrodites on back-crossing to C57BL/6[7].

That autosomal genes are involved in primary sex determination is also shown by the effects of an inherited dominant autosomal gene on chromosome 17, causing partial or complete sex reversal in XY individuals (Washburn and Eicher, in preparation; quoted in Eicher[5]).

Sex reversal may also be brought about by another Y-chromosome abnormality; an apparent rearrangement of the Y chromosome that leads in each generation to translocation of *Tdy* from the Y to the X chromosome during meiosis, in a manner probably analogous to that of *Sxr* transmission. However, in this case the translocation involves a very large piece of the Y, readily visible on karyotyping. XX individuals carrying one such translocated X chromosome are phenotypic males, whilst the reciprocal translocation gives rise to females with one normal X and a tiny translocation product lacking the *Tdy* gene[5].

It would appear that normal primary sex determination involves the *Tdy* gene located on the Y chromosome near the centromere, interacting with autosomal and perhaps pseudoautosomal (that is, X/Y linked) genes. Problems can arise through translocations of *Tdy* to other sites on the Y chromosome, especially to the distal sites which pair with the X chromosome during meiosis, since then transfer to the X chromosome is possible. *Sxr* and the translocation described by Eicher (see previous paragraph) probably arose in this way, and both result in the production of a normal X and an X that bears part of the Y. The autosomal genes that necessarily act in concert with *Tdy* also make possible abnormal sex determination due to improper timing of events which take place in the bipotential gonad (see Eicher[5]). Mutations, deletions and inappropriate alleles of such autosomal (and/or pseudoautosomal) genes might be involved.

It is still an open question as to how *Tdy* operates in this sequence of events, and whether its gene product is the male specific antigen which has been proposed as the differentiation antigen that causes testis differentiation. To complicate the matter further, it appears that there are two male specific antigens, one identified by T cells (the transplantation antigen, H-Y) and the serologically detected male (SDM) antigen. These two have been separated by testing mice with the sex chromosome constitution XO. These females, although H-Y negative[8] are SDM positive (ref 9, Koo, G. and Müller, U., personal communications). It has been argued[10] that neither of the two male specific antigens are candidates for the role of sole determinant of sex differentiation, since XO female mice are SDM positive but have ovaries, whilst XY^{pos} females are H-Y positive but have ovaries. The H-Y typing, using skin grafting and/or cytotoxic T cells, of a variety of mice whose karyotype is abnormal or unexpected, for example, XO (H-Y negative, female[8]), XX *Sxr* (H-Y positive, male[11,12]), XY^{pos} (H-Y positive, female[13]) is consistent with H-Y being tightly linked to *Tdy*, which is clearly not the sole testis determining gene. SDM typing is more problematical: from the XO data[9] the structural gene for SDM cannot be on the Y chromosome and is not *Tdy*. Moreover, SDM typing is difficult to reproduce and none of the SDM monoclonals tested so far have shown male specific binding (see ref.14 and Simpson & Goodfellow, unpublished).

By other less direct tests they are reported to have male specific activity[15],

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although at titers many orders of magnitude lower than that shown by monoclonals to other cell surface determinants that readily stimulate antibody production (for example, H-2, Thy-1, Ly antigens)[16].

In conclusion then, it appears that the differentiation of testis from the bipotential gonad, the key step that leads to the development of the male phenotype, is controlled by an interaction between autosomal genes and genes on the sex chromosome. Whether sex specific antigens are involved, and if so, how, is still a matter of speculation. □

Distant planetary satellites more accurately measured

from Ian Gatley

A BASIC step in the exploration of the Solar System is the determination of the sizes of planets, satellites and asteroids. Most of these objects are far too small for direct-imaging techniques from ground-based telescopes, but the cost of spacecraft makes size determinations from the ground extremely desirable. Fortunately, a simple and elegant method which combines the results of optical and IR observations is available, and two papers in this issue of *Nature* give new results for four of the uranian satellites (Uranus is to be visited by Voyager 2 in 1986), Neptune's largest satellite Triton and the most distant planet, Pluto.

The principle is this. Sunlight falling on the target is either absorbed or reflected; the energy absorbed is subsequently re-radiated in the thermal IR. Visual photometry measures the reflected component of the solar radiation and IR radiometry measures the absorbed component and the sum of these is equal to the total incident energy. One can therefore solve simultaneously for the albedo (reflectivity) and the size of the object under study (Morrison *Icarus* **19**, 1; 1973).

The principle is simple but its application is not, for two reasons: the IR signals are weak, and the atmosphere transmits poorly at the relevant IR wavelengths (around 20 μm) because of the presence of water vapour. These problems can be overcome by the use of a large IR telescope on a high dry site, such as the newly commissioned 3 metre NASA Infrared Telescope Facility on the 14,000 foot summit of Mauna Kea in Hawaii. This telescope was constructed primarily for the study of the Solar System, and its early results in this area are fascinating.

R. Hamilton Brown, Dale Cruikshank and David Morrison have measured the diameters and albedos of four satellites of Uranus — Ariel, Umbriel, Titania and Oberon, and find lower albedos and larger diameters than previous, less reliable estimates. Having made accurate size measurements, these workers now stress the importance of experiments to determine masses, for knowledge of the densities of the satellites will provide important insights into the origin and subsequent evolution of the uranian system. The albedo measurements give information on the surface composition of the satellites. Ariel, Umbriel, Titania and Oberon all have sizes similar to the large icy saturnian satellites, particularly Rhea, but they have significantly lower albedos, more like those found for Hyperion. It is noteworthy that Voyager images of Hyperion show it to be a reddish colour, suggestive of surface impurities.

Recently, further progress on surface composition has come from IR spectroscopy. Observations of Hyperion by Cruikshank and Brown (*Icarus* **50**, 82; 1982) have revealed the presence of water ice bands, which differ in detail from those seen on Rhea. Measurements of the dark side of Iapetus by L. Lebofsky, M. Feierberg and A. Tokunaga (*Icarus* **49**, 382; 1982) show an absorption feature thought to result from a hydrated silicate mixed with a small amount of water frost.

Stretching the capabilities of the size determination measurement to the outer Solar System, Morrison, Cruikshank and Brown have placed significant upper limits on the sizes of Triton, the major satellite of Neptune, and of Pluto. The results are consistent with icy objects of sub-lunar size, and with the hypothesis that the two objects are physically similar. The possibility that Triton is the largest planetary satellite can now be excluded as it is certainly smaller than the giant satellites Ganymede, Callisto and Titan. An improvement of only a factor of two in the quality of the IR measurements is likely to lead to positive detections, and hence definitive size measurements, for both Triton and Pluto.

The scientific value and cost-effectiveness of these Solar System studies are self-evident, and it is gratifying that NASA has reversed its earlier decision to stop funding for the Infrared Telescope Facility. The importance of IR astronomy for targets outside the Solar System is also gradually being realized in the study of star formation, galactic nuclei and many other dusty interstellar environments. It is to be hoped that the facility will long continue to exploit the special advantages of Mauna Kea, where, as well as University of Hawaii telescopes, there are now two international observatories in operation; the Canada-France–Hawaii Telescope (CFHT) and the United Kingdom Infrared Telescope (UKIRT). More telescopes are planned for the future, including the SERC Millimetre Wave Telescope. □

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100 years ago

POLLUTION OF THE ATMOSPHERE

THERE was a letter in *Nature* some time since, calling attention to the pollution of the atmosphere by the burning of coal; and it was calculated that in the year 1900, all animal life would cease, from the amount of carbonic dioxide; but the author had overlooked the fact that the rain is continually cleansing the atmosphere of this, and the fall of this rain on the ground, and the combination of this with various salts; besides the oceans alone would absorb their own bulk at normal pressure, but at an increased pressure of, say half a mile deep, would dissolve more than we are likely to need for hundreds of years.

But there are other products of combustion, or rather of incomplete combustion, that are not brought down in this manner by rain, as hydrogen and the hydrocarbons, chiefly marsh-gas and ethylene.

Since the year 1854 (as near as I can estimate) there has been burnt 10,000 million tons of coal; and if we say (in its consumption by household grates, leakage by gas-pipes, etc.) 1-100th escapes, then 100 million tons of hydrogen and hydrocarbons are floating in the atmosphere or 1-10,000,000 part in bulk; if we say the average proportion of hydrogen to be ·45, and of marsh gas ·35, and of ethylene ·4, we have ·84 per cent of gases that are lighter than air, and it is more than probable that the law of diffusion of gases, as demonstrated with jars, does not apply to the atmosphere. The cases are not parallel: in the air we have unconfined space, pressure, and temperature diminishing infinitely, conditions favourable to the lighter and the gas with the greater amount of specific heat rising and maintaining its elevation, especially as we know that in *large* halls carbonic dioxide is found in larger quantitites on the floor. According to Prof. Tyndall's researches, hydrogen, marsh gas, and ethylene have the property in a very high degree of absorbing and radiating heat, and so much so that a very small proportion, of only one thousandth part, had very great effect. From this we will have a marked influence on the climate of the world. The mountainous regions will be colder, the Arctic regions will be colder, the tropics will be warmer, and throughout the world the nights will be colder, and the days warmer. In the Temperate Zone winter will be colder, and generally differences will be greater, winds, storms, rainfall greater.

H. A. PHILLIPS

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REVIEW ARTICLE

Giant voids in the Universe

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Recent observations indicate that most galaxies are concentrated in superclusters consisting of galaxies, and clusters of galaxies, aligned along strings. Giant volumes exist between superclusters which are almost empty of visible objects. Theories of galaxy formation predict the formation of non-spherical superclusters and giant voids. Large-scale structure changes very slowly, so the currently observed structure reflects the whole history of galaxy formation and structural evolution.

OBSERVATIONS reveal that galaxies are distributed more or less randomly, showing a moderate tendency towards clustering which statistically can be expressed in terms of the galaxy correlation function[1]. It has usually been assumed that field galaxies and clusters of galaxies are pretty well isolated from each other (island universes), and the correlation function was thought to reflect the size of clusters and the deviation from a purely random distribution.

Modern detectors have made it possible to determine the redshifts of many galaxies. The redshift, interpreted as the expansion speed of the Universe, is proportional to the distance of the galaxy from the observer. Combined with the apparent position in the sky, it is therefore possible to derive a three-dimensional distribution of galaxies in space.

The results of recent studies of the three-dimensional distribution of galaxies are surprising. Most (90%) galaxies are concentrated in strings, groups and clusters of galaxies[2–6]. Neighbouring strings form a connected network which can be called superclusters[3]. Superclusters are often flattened and fill only a small fraction of the total space (<10%), the remaining space between strings and superclusters contains almost no luminous matter[3,6]. The diameter of voids between superclusters reaches 100 h^{-1} Mpc, where h is the Hubble constant in units of 100 km s^{-1} Mpc^{-1} (refs 3, 7), and such voids appear to make up >90% of the total volume of the Universe.

This picture, particularly the presence of giant voids, differs completely from earlier expectations and raises several questions. First, is a revision needed of the fundamental cosmology, the Friedmann–Lemaitre model of the Universe? Second, what does the observed structure tell us about the process of galaxy formation and about the very early Universe?

Observed random velocities of groups and clusters relative to the neighbouring string network seem to be small, of the order of 100 km s^{-1} (ref. 6). During the whole Hubble time a galaxy with such a small velocity could travel a distance <1 Mpc. Although the cluster network as a whole may have larger anomalous velocities[1,8,9], this does not alter the conclusion that the structure of the Universe changes slowly. Thus the present structure reflects the history of its formation and evolution.

We now review new observational data, and pay special attention to quantitative methods of analysing this data. Thereafter a scenario explaining the structure formation is outlined and competing scenarios are discussed.

All current theories of the formation of the structure are essentially gravitational clustering theories based on the theories of the expansion of the Universe[10] and of density fluctuations[11]. Both theories were first formulated in the framework of general relativity. Their essentially newtonian content was understood much later[12].

Geometry of galaxy distribution

To a first approximation the clumpiness of galaxy distribution can be expressed in terms of a correlation function[1]. The most popular two-point correlation function says, however, practically nothing about the characteristic pattern of galaxy distribution.

So far galaxy systems have been described only qualitatively. Our conviction that systems of galaxies of various types (groups, clusters, cluster chains) do exist is based on visual inspection of photographs and on galaxy counts. To build up quantitative theories of how the structure of the Universe formed, its structure must also be described quantitatively.

One possible way to do this is to use the so called 'cluster analysis' (see, for example, ref. 13). Draw a sphere (in the two-dimensional case a circle) of radius r around each dot representing galaxies. If the radius r is small enough, encircled regions are well separated from each other and the whole space is divided into isolated 'islands' containing galaxies and a continuous 'ocean' of empty space. When we take a larger radius, several neighbouring 'islands' join to form connected systems of galaxies. With an even larger radius, most galaxies join to form large connected systems. At a very large radius practically all galaxies join to form a single connected system.

The neighbourhood radius r at which galaxies form connected systems depend on the density of galaxies in space. With a small radius only the densest systems, cores of groups and clusters of galaxies, are picked up. Then, with increasing radius r neighbouring groups and clusters join to form elongated double or multiple clusters, thereafter still larger and less denser aggregates of galaxies will form connected systems.

The form and orientation of connected systems, their length, total volume and other properties depend on the pattern of the galaxy distribution.

Distribution of galaxies in space

Our analysis of the three-dimensional distribution of galaxies in space is based on a catalogue of all available redshifts of galaxies compiled by J. Huchra (Harvard-Smithsonian Center for Astrophysics). In the northern sky the catalogue is complete up to apparent magnitude 14.5, in the southern sky up to apparent magnitude 13.0. For galaxies brighter than the knee-point of the luminosity function (−21.0 absolute magnitude)

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the catalogue is complete up to distances 130 and 65 Mpc (northern and southern sky, respectively). Throughout this review we use the Hubble constant $H = 50\ \mathrm{km\ s^{-1}\ Mpc^{-1}}$. Due to strong galactic absorption it is difficult or impossible to observe external galaxies in areas close to the Milky Way. Here, of course, data are not complete.

Several slices through the local supercluster are shown in Fig. 1, where supergalactic rectangular coordinates are also indicated and long galaxy strings between the Virgo and Coma clusters and regions void of galaxies can be seen.

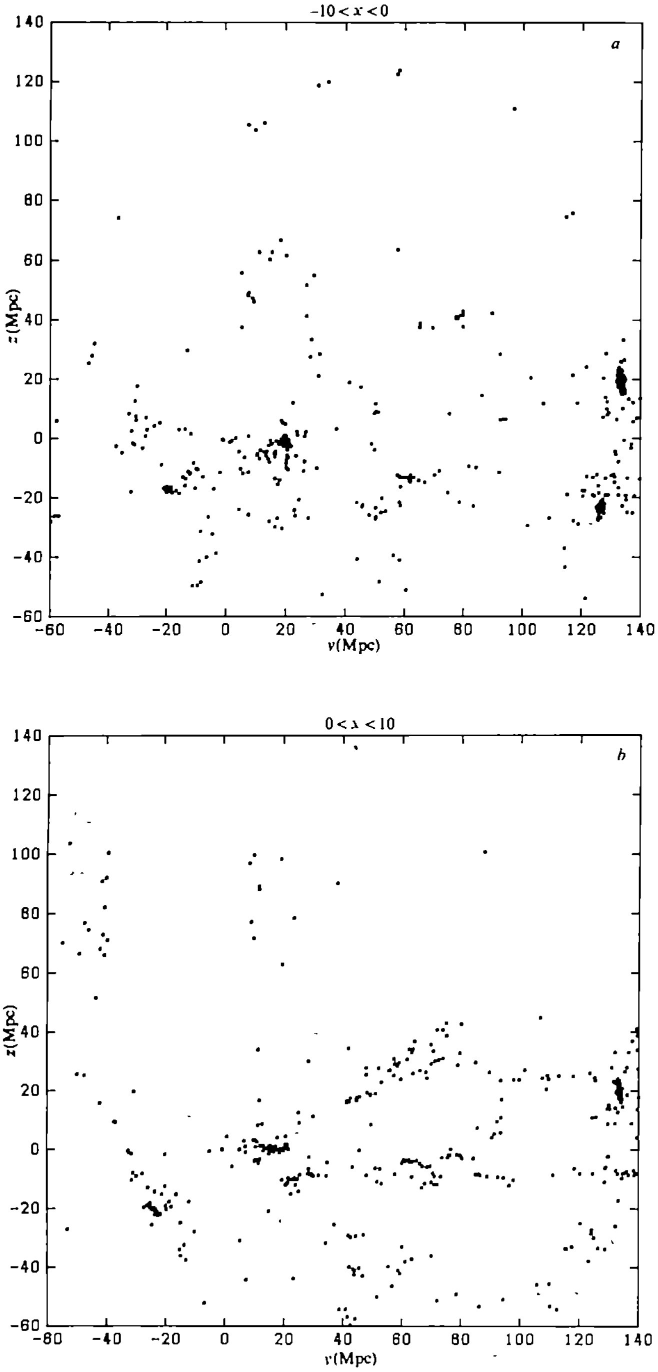

Fig. 1 Slices through the Local supercluster. Supergalactic rectangular coordinates are shown: the x and y axes lie in the supergalactic plane. The Galaxy is at the centre, the Virgo cluster lies in the y axis direction at $y = 20$ Mpc. Bright clusters of galactics at $y = 130$ Mpc are the Coma cluster ($z = 20$ Mpc) and A1367 ($z = -10$ Mpc). Galaxies brighter than −19.5 absolute magnitude have been plotted.

Properties of systems of galaxies have been studied using the cluster analysis. For comparison, identical analysis was carried out for several model catalogues. One model was calculated following the method of Soneira and Peebles[14]. The second model was generated for a full random (Poisson) distribution of galaxies. The third model was taken from a N-body experiment, based on the adiabatic scenario of structure formation[15]. These model catalogues H, P, and A have approximately equal number and mean density of objects. The two-point correlation function was calculated for all model catalogues and for the observed (O) catalogue (the region centred at the Virgo cluster, cube side of 80 Mpc, limiting absolute magnitude −19.5, total number of galaxies 866). The correlation function is plotted in Fig. 2. Functions are close to each other and only for the random P-distribution do we have zero correlation, as expected.

The maximal length of connected regions as a function of neighbourhood radius r is plotted in Fig. 3 for the observed and model distributions. At small r all curves corresponding to catalogues with normal correlation function have similar slopes. Connected regions are round or slightly elongated. Most regions found from the observational data correspond to know groups and clusters of galaxies.

At a certain critical radius (~1 Mpc) the slope of O and A curves abruptly increases. This corresponds to the transition from single to multiple clusters and cluster chains. With growing radius the length of chains rapidly increases and at some other critical radius (~5 Mpc in our case) it exceeds the size of the cube under study.

For the hierarchical H-catalogue the length increases slowly over the whole neighbourhood radius interval studied. For the P-catalogue the length increases at small r very slowly (the catalogue contains no built in systems), but then the slope gradually increases.

Figure 4 shows the distribution of the number of galaxies in systems of different multiplicity for the observed and model catalogues. All histograms correspond to an neighbourhood radius $r = 5$ Mpc.

At this radius about half of all galaxies in the O and A catalogues form a single connected system. H and P catalogues have no large single connected systems. Galaxy systems, not connected to a single supersystem, have multiplicity <64. In the case of the O catalogue these galaxies are more or less evenly distributed among systems of different multiplicity. The H catalogue has too few singles and pairs (in comparison with the O catalogue). The distribution of small systems in the A and P catalogues is similar, peaking at singles.

Figures 3 and 4 can be interpreted as follows. Both random catalogues, H and P, behave completely differently from the observed O catalogue. Thus the observed distribution cannot be considered as a simple hierarchical one. In general, the A distribution has similar properties to the observed one. But we note two important differences.

First, in the A catalogue test particles that are not connected to the single large connected system, are distributed evenly like the particles in the P catalogue which can be considered as a field population. The O catalogue has no appreciable field population. Thus in regions of lower than the average matter density no galaxy formation takes place. The comparison of respective numbers of test particles suggests that about half of primordial matter can still be found in low density regions in some pre-galactic form.

The second difference lies in the fact that the A catalogue has no systems of intermediate size and mass. The observed catalogue has at this neighbourhood radius one large system (containing half the galaxies) and about 10 systems containing galaxies of multiplicity between 8 and 64 with length from 10 to 35 Mpc (mean value 20 Mpc). These systems are strings of galaxies and groups of galaxies, they contain some poor Zwicky[16] clusters but no rich Abell[17] clusters. The presence of systems of intermediate scale demonstrates that the Universe has some fine structure, which is lacking in the A model.

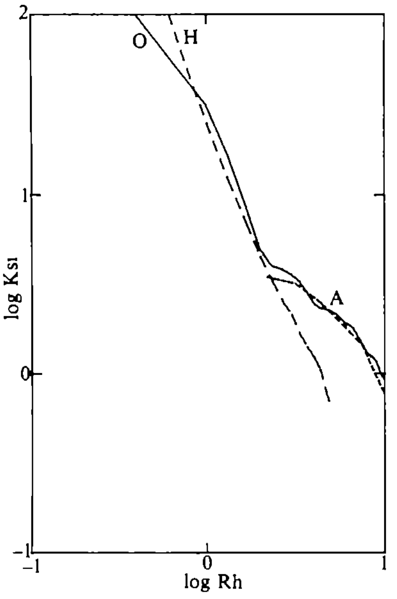

Fig. 2 Correlation function for the observed catalogue (O) around the Virgo cluster (cube of side 80 Mpc), for the catalogue generated by Soneira and Peebles method (H) and for the adiabatic model (A) (ref. 61).

The status of galaxies, not connected to systems of large or intermediate scale at this neighbourhood radius, is not clear. There are two possibilities, either these galaxies and small groups are outlying members of larger systems, or they constitute a true field population. To check these possibilities we have studied galaxies in a smaller and more complete region around the Virgo cluster. It is a cube of side 40 Mpc, has a limiting absolute magnitude of −16.5, and contains 1,009 galaxies. We have calculated the distribution of galaxies among systems of various multiplicity for several values of the neighbourhood radii (1–5 Mpc). The results indicate that the relative number of singles and small systems quickly decreases with increasing radius, for $r = 3$ and 4 Mpc only 10 and 5% of all galaxies are located in systems of multiplicity <8, respectively. This test emphasizes that most small systems are outlying members of larger systems. The field population, if it exists, does not exceed 5–10% of the total number of galaxies.

Let us now estimate the volume occupied by systems of galaxies. Galaxy strings can be approximated by cylinders of length, found from the previous study of connected systems, and of radius, equal to the characteristic radius of galaxy groups and clusters. Nearby rich clusters of galaxies have following radii: Virgo, 2.2 Mpc; Perseus, 2.9 Mpc; Coma, 2.5 Mpc. The radius of groups found in our study of connected systems lies between 0.5 and 2 Mpc, the r.m.s. radius being 1.0 Mpc. We adopt as a mean outer radius of the cylinder $r = 2$ Mpc. The total length of galaxy strings in cubes around the Virgo cluster is 140 Mpc and 380 Mpc (smaller and larger cube respectively), so we found that within these cubes galaxy strings occupy 0.110 and 0.037 of the total volume.

To estimate the volume filled with galaxy systems in a larger region, only data on the distribution of bright galaxies (absolute magnitude <−21) can be used. For this we have divided the region under study into cubic cells of various size. We note that at cell size 5 Mpc relative volume of cells occupied by bright galaxies is approximately equal to the relative volume occupied by galaxy systems. Using this method we have found that the filling factor of a cube of side 200 Mpc centred on the Virgo cluster is only 0.01. Thus the density of galaxies outside superclusters should be very small.

Where are the low density areas? Figure 1 shows that at high supergalactic z coordinates the galaxy density is much lower than near the supergalactic plane $z = 0$. The almost complete absence of galaxies in front of the Hercules supercluster is well known[5]. To study the galaxy distribution in vertical supergalactic direction we have found the number of bright galaxies in rectangular sheets of sizes 200×200×20 Mpc. One sheet is centred at the Virgo cluster, others lie upwards and downwards. The distribution of numbers of galaxies in these sheets as a function of z is given in Fig. 5. We see that the number of galaxies rapidly decreases with increasing distance from the supergalactic plane. A similar distribution in the x and y directions shows no systematic trend.

The rapid decrease of the number of galaxies in the z direction is partly due to the galactic absorption: the zone of avoidance crosses the sky near supergalactic poles. A rough estimate shows that the number of galaxies at extreme z values may be underestimated by a factor of three. The corrected distribution is also plotted in Fig. 5, as well as the distribution of rich clusters of galaxies having redshift <7,000 km s^{-1}. Nearby clusters of galaxies are very strongly concentrated towards the supergalactic plane and belong to three well studied superclusters: the Local, Coma, and Perseus, as well as to several other previously unrecognized superclusters.

This sheet of superclusters can be considered as a wall shared by two local cells of the Universe, the northern and the southern[18]. The Hercules supercluster, the supercluster around Abell cluster A539 and several southern superclusters form side walls of these cells. The far side of the local cells can be located only approximately, due to the lack of observations near the galactic plane. Both local cells have a diameter of ~200 Mpc.

Available data suggest that the interior of local cells contains no rich clusters of galaxies. In this respect they are similar to cells found by Jõeveer *et al.*[2] behind the Perseus supercluster and by Kirschner *et al.*[7]. However, there are also some poor Zwicky[16] clusters here[19]. Our study confirms the results obtained by Balzano and Weedman[20] indicating that Kirshner's void is not completely empty. Zwicky clusters as well as galaxies located in cell interiors are not randomly spaced. As in superclusters, galaxies and clusters form long strings here. The basic differences between galaxy strings in superclusters and in cell interiors lie in the population and the spacing: in cell interiors

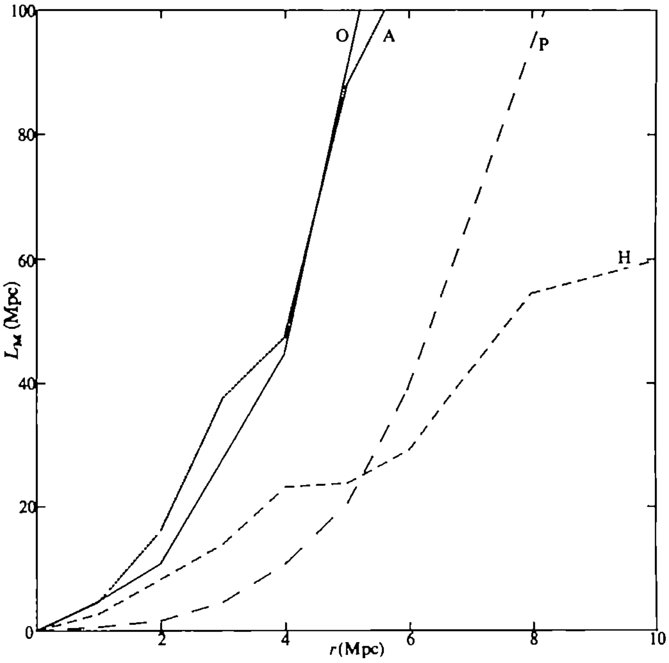

Fig. 3 Maximal length L_M of connected regions as a function of neighbourhood radius r for four catalogues[61].

the string population is smaller and the spacing between neighbouring strings is larger.

From available data the luminosity density in different structures can be calculated. As luminosity indicators we use bright galaxies. The total luminosity in solar units for standard luminosity functions is

$$L = n^* \times 3.6 \times 10^{11} L_\odot$$

where n^* is the number of galaxies brighter than $M = -21.0$ (ref. 2). We found that for the average luminosity density $5 \times 10^7 L_\odot \mathrm{Mpc}^{-3}$, the mean density in cell interiors is about 3 times lower, but in galaxy strings about 70 times higher. An upper limit for the luminosity density outside connected regions is 10 times lower than the average luminosity density. Thus the luminosity density contrast between galaxy strings and 'empty' space is at least three orders of magnitudes.

Cluster analysis gives us the possibility of picking up and studying individual connected systems. This study demonstrates that most systems are elongated and look like cluster strings in the Perseus supercluster[2,3]. In some cases, however, small sheets of galaxies have been found; one such sheet is connected with the Virgo cluster. Three orthogonal views of this system are given in Fig. 6. The interpretation of sheets of this type is still open. No large sheets of galaxies have been found so far.

Compatibility of the observed structure with the Friedmann–Lemaitre model

Large voids between superclusters have a volume of $\sim 10^6 \mathrm{Mpc}^3$ (refs 3, 7). In an expanding Universe the diameter of voids expressed in redshift units remains approximately constant (exactly constant in the case of a non-decelerating Universe), thus we can adopt as a first approximation $\Delta z = 0.05$. Let us assume that the cellular structure was formed at $z = 3$–10, as indicated by a comparison of densities in various structural elements and by the cutoff of quasar redshifts at $z = 3.5$ (refs 3, 21). If this is the case, then within the horizon our Universe should contain $\sim 10^6$ cells.

There is little or no evidence for the presence of still larger structural units in the Universe. Cell dimensions in comparison with the Universe as a whole are still small, thus we see no need for a revision of the fundamental principles of cosmology. Our confidence in the classical model is strengthened by small temperature fluctuations of the microwave radiation, indicating small density fluctuations at the epoch of decoupling of radiation and matter ($z \approx 1{,}300$) (refs 22, 23).

How the structure of the Universe formed

At present it is difficult to infer the type, the spectrum and the amplitude of initial perturbations from general physical principles. So theorists consider all possibilities. The most developed scenarios of the formation of the large-scale structure are based on two different assumptions about the character of perturbations in the pre-decoupling era[24].

First, at an early stage both matter and radiation density were perturbed so that their ratio was constant in space and time. Because this ratio is conserved in adiabatic processes such perturbations are termed adiabatic.

Second, it is supposed that the matter density was perturbed but the radiation density was not. These perturbations are known as isothermal since in this case temperature is assumed to be the same everywhere.

Both scenarios have important features in common: the initial perturbations on scales larger than galaxy ones are small, the large density contrast and the large-scale structure develops due to the gravitational forces. In an expanding Universe small density fluctuations can grow only if gravitation amplifies them. In this sense both the adiabatic and the isothermal scenarios are gravitational clustering theories, however, the principal differences between them lie in the scale of post-decoupling perturbations.

Adiabatic scenario

Adiabatic fluctuations on scales smaller than a characteristic mass $M_c \approx 10^{13}$–$10^{15} M_\odot$ were damped before decoupling due to photon viscosity[25]; after decoupling one finds practically no fluctuations on scales smaller than this limit. Perturbations of the scale M_c had the largest amplitude, thus under- and overdensities of this scale developed first. Galaxies and clusters of galaxies formed in dense regions after the gas collapsed and cooled. The formation of dense regions and galaxies started at a relatively late epoch $z = 3$–10.

The principal feature of the adiabatic scenario lies in the fact that the formation of large-scale units occurs in a cold environment. The gas pressure is negligible until the final stages of formation of large-scale structures. Numerically this fact is expressed in terms of the ratio of the Jeans mass of the neutral gas after decoupling, $M_J \approx 10^6 M_\odot$, to the characteristic mass of adiabatic perturbations, $M_c \approx 10^{13}$–$10^{15} M_\odot$: $M_J/M_C \approx 10^{-7}$–10^{-9}. In the first approximation we can neglect the temperature and gas pressure, assuming $M_J/M_C = 0$.

The evolution of perturbations in a pressureless environment is now well established. Before giving more details on the formation of the large-scale structure let us outline the main processes leading to galaxy formation in the adiabatic scenario[24,26,27].

Very flattened clouds—'pancakes'—with masses about $M_C \approx 10^{13}$–$10^{15} M_\odot$ were formed first[28]. At its arrival to a pancake the gas is heated and compressed in the shock wave, which represents the boundary of the pancake. Inner parts of pancakes cool quickly due to radiative losses of their thermal energy causing an increase in density[29]. So in inner parts of pancakes the conditions become favourable for galaxy formation[26,27]. Shock waves on the boundaries of pancakes favour the generation of rotation, thus spiral galaxies could form easily[30]. The general trend in the evolution on galaxy scales after pancake

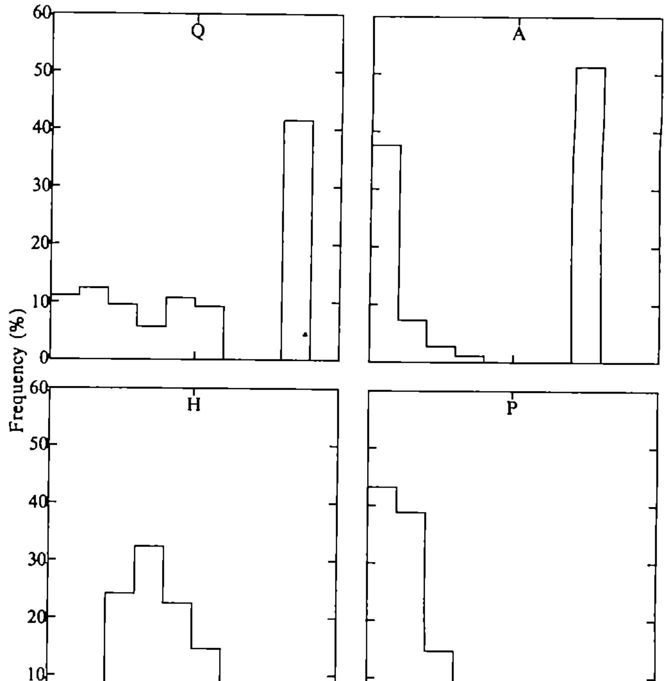

Fig. 4 Distribution of galaxies according to the multiplicity of the system. Neighbourhood radius $r = 5$ Mpc. Multiplicity is expressed in powers of 2 (5 corresponds to $2^5 = 32$ and so on)[61]

formation is the fragmentation of pancakes into smaller units. Thus the adiabatic theory is often called the fragmentation scenario.

Large-scale structure and the catastrophe theory

We now consider the formation of larger-scale objects. We believe that the processes of galaxy formation do not have a major influence on the motion of matter on scales of rich clusters and superclusters. As noted earlier, the growth of adiabatic perturbations has one important property: the evolution proceeds at low temperature of self-gravitating medium. This evolution has a simple approximate analytical solution[28]. As the smooth initial perturbations develop into the nonlinear stage, dense regions will be built up by the concentration of matter into caustics—the intersection of particle trajectories.

The formation of caustics in a swarm of dust particles has been demonstrated by Lifshitz and Khalatnikov[31] and later studied by Grishtshuk[32]. Initially it was thought that this case is physically not realistic because an arbitrarily small pressure removes sharp caustics. However, the idealized case is a very good approximation to the actual situation. The geometry of dense regions is closely related to that of caustics in a pressureless medium.

Geometrical properties of caustics can be studied mathematically using the catastrophe theory[33,34]. Because adiabatic perturbations are potential (rot $\vec{v} = 0$), the study of singularities in the potential flows is of great interest.

As shown by Arnold and co-workers[35] following an early suggestion by Zeldovich[28], pancake-like singularities known in the catastrophe theory as lips formed first. At a later epoch pancakes grew, intersected and formed a fairly pronounced cellular structure[36]. Matter continued to flow towards and within pancakes, so that some time later higher-order singularities, lines and knots, formed. All this happened in a similar way both in gaseous medium and in some collisionless cold (neutrino) medium. This phase ended with a cellular structure of high density sheets, strings in sheets and knots connected by strings. At this stage dense regions fill $<10\%$ of the total volume of the Universe, while isolated low density regions (cell interiors) fill the remaining major part of the volume of the Universe.

Numerical experiments demonstrate[15,37] that the density is highest in knots (zero-dimensional structures), high in strings (one-dimensional structures) but much lower in sheets (two-dimensional structures). Subsequent evolution of the structure depends on three processes, the formation of galaxies, the gravitational redistribution (clustering) of galaxies, and the expansion of the Universe.

One comment is necessary here: galaxies are easier born in dense and cold gas. Thus it is natural to expect that no galaxies will be formed in cell interiors and that an intensive galaxy formation would occur along the lines and in the knots. It is not evident whether the density in all sheets is sufficient for galaxy formation or not. Perhaps some gaseous sheets remain in gaseous form and can be seen as absorption lines in quasar spectra[38,39].

Later gravitational clustering tries to destroy the cellular structure, the rate of which depends on the mean density of the Universe. In a low density Universe cellular structure is maintained for a long time, in a closed Universe this structure disappears in a time scale comparable with the Hubble time, thereafter galaxies clump to clusters of very high mass[15,37].

Neutrino dominated Universe

Perhaps the weakest point in the adiabatic scenario is its need for too large an amplitude of density perturbations at the decoupling era: $\delta\rho/\rho \approx 10^{-3}$ if $\Omega = 1$ and $\delta\rho/\rho \approx 10^{-1}$ if $\Omega = 0.02$ (ref. 40). As noted already by Silk[23], density fluctuations at the epoch of decoupling correspond to similar angular fluctuations of the temperature of the microwave background, $\delta T/T \sim 1/3\delta\rho/\rho$. On the other hand, observations give an upper limit of temperature fluctuations of the order 10^{-4} (refs 22, 23).

In an open Universe density fluctuations grow extremely slowly. To obtain the observed density contrast, it is also necessary to have at the epoch of decoupling high-density perturbations in contrast to small temperature fluctuations of the microwave background.

One can infer from these arguments that $\Omega = 1$ is necessary for the adiabatic scenario. However, the data on helium and deuterium abundances strongly support the model of a low baryon density. Deuterium cannot be produced in stars like heavy elements, the whole deuterium observed now must have originated during the primordial fireball stage of the Universe. Deuterium production rate is extremely sensitive to the density of baryons in the Universe. Observed deuterium abundance suggests the baryon density 0.01–0.1 of the closure density[41], that is the Universe should be open by a large margin.

Last but not least, flat rotation curves of galaxies, the dynamics of double galaxies and of clusters of galaxies indicates that giant galaxies and clusters of galaxies are embedded in huge invisible coronas[42,43]. The mass of the coronas exceeds the mass of visible galactic populations by a factor of 10 or more.

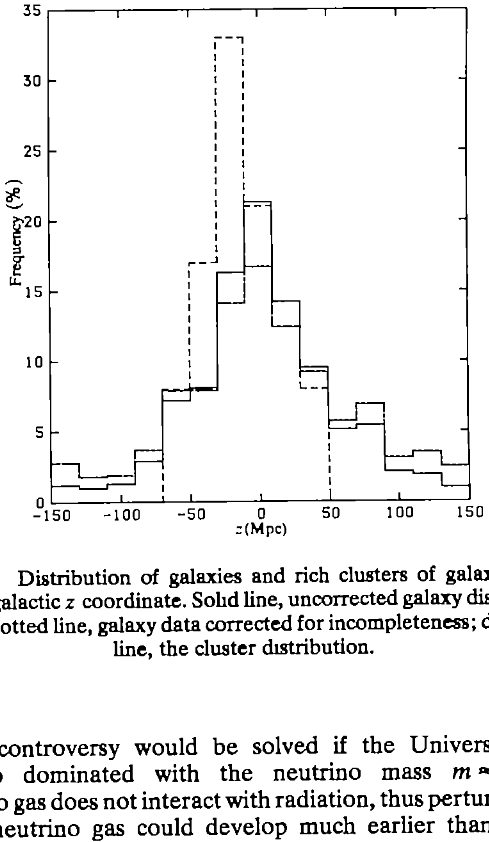

Fig. 5 Distribution of galaxies and rich clusters of galaxies in supergalactic z coordinate. Solid line, uncorrected galaxy distribution; dotted line, galaxy data corrected for incompleteness; dashed line, the cluster distribution.

This controversy would be solved if the Universe were neutrino dominated with the neutrino mass $m \approx 10$ eV. Neutrino gas does not interact with radiation, thus perturbations in the neutrino gas could develop much earlier than in the baryon dominated Universe and could have the necessary amplitude. Baryon gas is bound to radiation and has smaller density fluctuations, after decoupling it simply flows to gravitational wells formed in the neutrino gas.

Thus in the neutrino dominated Universe one has low baryon density $\Omega_b \approx 0.01$–0.1 while the total density is close to the closure once $\Omega_t \approx \Omega_\nu \approx 1$.

The formation of the structure in a neutrino dominated Universe is, essentially, an adiabatic scenario[44–51]. The initial ratio of baryons to neutrinos is the same everywhere (the entropy is constant), small-scale fluctuations are damped, the characteristic mass of objects to form first is $10^{15} M_\odot$ as in the conventional adiabatic scenario.

However, the structure of pancakes is more complicated: inside a neutrino pancake there is a pancake made of baryons which is similar to that described earlier.

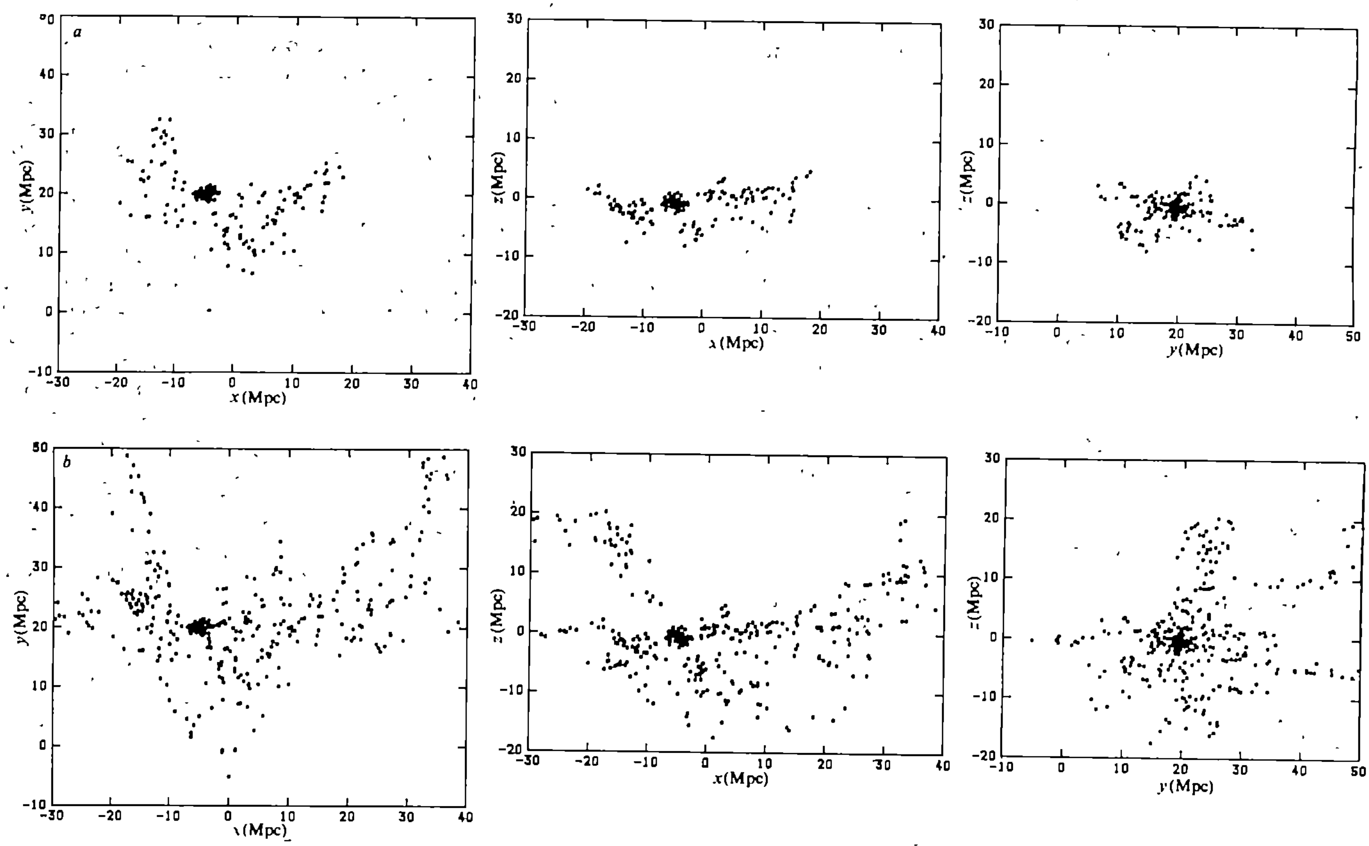

Fig. 6 Three views of a connected system around the Virgo cluster[61]: *a* corresponds to the neighbourhood radius $r \simeq 4$ Mpc; *b* to the radius $r = 5$ Mpc. *a* shows two sheets of galaxies in both sides of the Virgo cluster, *b* demonstrates the presence of a string network around the cluster.

Isothermal scenario

According to the isothermal scenario, small-scale density fluctuations were not damped. Thus, it is natural to think that the mass of the first objects to be formed was limited by the gas pressure—by the respective Jeans mass after decoupling 10^5–$10^6 M_\odot$ (refs 49, 52). Thus globular cluster-sized objects were the first primordial building units formed soon after decoupling, $z \simeq 10^3$. During the subsequent evolution larger building blocks, galaxies and clusters of galaxies, were built up step-by-step by gravitational clustering.

The formation of the first bound objects of mass $10^6 M_\odot$ takes place in the environment when one cannot neglect the influence of pressure. Therefore they must have a shape that is almost spherical. Part of gravitational energy liberated during their formation transforms into chaotic motion, having the role of 'temperature' of the 'gas' of these primordial objects. Hence, according to the isothermal scenario, the formation of large-scale structural units proceeds in a high-temperature 'gaseous' environment. The 'gas' pressure was active during the whole process of the formation of the structure so that it is difficult to build up strongly anisotropic structures.

Extensive series[53–55] of three-dimensional N-body calculations in the framework of the isothermal model reproduced the observational correlation function. The general appearance of particle distribution was claimed to be similar to the observational distribution of galaxies. However, it seems that in details the calculated distribution is more amorphous than the observational one.

Tepid and other cosmological models

Another scenario of the formation of the large-scale structure is suggested by Ostriker and Cowie[56]. They argued that the formation of large-scale second generation objects (superclusters around voids) may be due to the nuclear energy released during the explosions of first generation objects. These explosions can give rise to expanding shock wave shells which ignite star and galaxy formation in the remaining gas.

Earlier it was recognized that total energy released during explosions of primordial objects is comparable with the energy contained in the microwave background radiation. This point has led Rees (refs 57, 58 and references therein) to the hypothesis that microwave radiation can be of nuclear origin and thus that there is no need for a hot Big Bang.

Both scenarios predict many dead remnants of first generation stars, therefore, they easily solve the problem of missing mass.

Observable differences expected in different formation scenarios

In the isothermal case dominant post-decoupling perturbations had a scale corresponding to a mass of $10^6 M_\odot$. Star cluster like objects formed very quickly after decoupling and most of the primordial gas went into stars at an early stage. Large-scale structures, such as superclusters and large voids, were due to large-scale perturbations, which needed more time to develop and formed gradually. Galaxies should have a tendency towards clustering, but could be found everywhere between clusters and strings. Strings should be short and not connected with other structural elements, voids should have moderate sizes. These expectations have been confirmed by numerical simulations[53,54].

In the adiabatic case small-scale perturbations were damped. The Universe evolved slowly and was gaseous for a long time. In contrast to the isothermal scenario, clusters and groups of galaxies formed at a later epoch along the initially continuous pre-existing network of gaseous strings and knots. The density of gas outside this network was small, thus no galaxies should have been formed there[36,59]. Some ionized gas and dark matter is still in 'empty' areas, because it is difficult to evacuate these large regions completely[60]. This gas should have had primoridal abundance.

In the Ostriker and Cowie[56] version one expects to have rather empty cell interiors, also filled with some ionized gas that should have a normal chemical composition.

The expected differences are of two types: (1) the composition of the gas outside superclusters; and (2) the fine structure of superclusters and 'empty' areas.

Gas layers in cell walls can be studied using absorption line spectra of distant quasars as suggested by Oort[38] (see also ref. 39). Here not only the presence of gas but also its chemical composition is important. Geometrical properties of alignment of galaxies and clusters of galaxies can be best studied in the vicinity of our own Galaxy.

Problems

The history of the formation of large-scale structure critically depends on basic cosmological constants, the time scale available (Hubble constant H), the overall density of matter (the ratio of density to the closure density $\Omega=\rho/\rho c$), and last but not least, on the nature of the dark matter (is it baryon matter or neutrino gas?).

A very important observational constraint is the temperature fluctuation of the microwave background radiation. Here not only the upper limit but also the actual fluctuations and their angular size are needed.

Conventionally observational studies in cosmology are based on data of objects located at high 'cosmological' redshifts. The study of superclusters in our vicinity has shown that important cosmological information can be received also by studying the distribution of nearby galaxies. Here we can study the distribution in greater detail.

To build up a quantitative theory of the formation of the structure in the Universe, the structure itself must be characterized quantitatively. Some weak points of principal structure formation scenarios that are already known are now considered.

The adiabatic scenario predicts the formation of structures of three types: surfaces, lines and dots. Numerical experiments suggest that the density is highest in dots, relatively high in lines, much lower in surfaces and very low in regions outside these singularities. Were galaxies formed in all of dense regions? If so, should we expect to observe all three kinds of galaxy system: pancakes, strings and clusters? The tests carried out so far demonstrate the presence of strings and clusters, the evidence for pancakes is controversial. Does this indicate that galaxy formation occurs basically at the structures having highest densities—in lines and dots?

The adiabatic scenario also predicts the formation of a simple cellular structure[36]. Observations indicate that the real distribution is much more complex. Rich clusters of galaxies (of the type included in the Abell catalogue) and connecting cluster chains surround regions, which are void of rich cluster. Voids of this type have a diameter of 200 Mpc. These voids are not completely empty, they are intertwined by a lattice of galaxy strings. Near the rich cluster the network of galaxy strings is much more closely spaced. How can we explain this hierarchical structure? Why do the properties of the fine structure (small galaxy strings) depend on the large-scale environment (proximity to rich clusters)?

The severest problem encountered by the isothermal scenario is the amorphous character of the calculated structure. This conclusion is based on the visual inspection of the results of N-body calculations. To draw more quantitative conclusions, one must await objective checks.

Another weak point in the isothermal scenario is the galaxy formation. As Silk has pointed out[46], there are several arguments (such as high central concentration demanding dissipation of extra energy, and radial gradient of chemical composition favouring *in situ* formation of heavy elements) demanding that galaxies have been formed directly from gas, not by clustering of ready stellar systems of lower mass.

Astronomers usually question which formation scenario is right. But both basic scenarios have weak points—perhaps it would be more correct to ask, which physical processes have led to the formation of structure in the Universe?

To answer this question, more observational data are needed. In particular, the study of 'empty' regions is important. So far astronomers have concentrated on rich clusters and other prominent features. The study of empty regions requires the coverage of large areas in the sky up to a fairly faint apparent magnitude, and thus much observing time. The first line of attack should be the study of the central regions of local cells. This can be done using telescopes of moderate size (of the 1.5–2 m class).

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ARTICLES

Absolute age of formation of chondrites studied by the ^{87}Rb–^{87}Sr method

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The ^{87}Rb–^{87}Sr isochrons for H-, E- and LL-, chondrites are undistinguishable from each other. The joint isochron yields an age of 4,498 ± 15 Myr if the rubidium decay constant $\lambda\ ^{87}Rb = 1.42\times10^{-11}\ yr^{-1}$, and a strontium initial ratio $^{87}Rb/^{87}Sr = 0.69885 \pm 0.00010$. For the age to agree with U–Th–Pb data, $\lambda\ ^{87}Rb$ would have to be changed to $1.402 \pm 0.008\times10^{-11}\ yr^{-1}$.

DATING of meteorites helps to decipher the mode of formation and the history of these extraterrestrial objects. This also provides constraints—mainly as a starting point—for the evolution of the terrestrial planets. In this respect, chondrites are particularly useful because they are chemically undifferentiated relative to the solar abundances and have had a fairly simple history. Thus it is thought that their age of formation corresponds to that of the Solar System. An absolute value for this age can in principle be obtained from the radioactive chronometers and we aim here to give a 'best' ^{87}Rb–^{87}Sr age of chondrites and discuss its interpretation.

In this method, the ^{87}Sr/^{86}Sr isotopic ratio of a series of objects is plotted against their ^{87}Rb/^{86}Sr atomic ratio. If these objects have been formed simultaneously with the same ^{87}Sr/^{86}Sr initial isotopic composition, and if they evolved since as closed systems (without external exchange of Rb and Sr), they should define a straight line in such a diagram. This line is called an isochron; its slope yields the age of the objects and its intercept gives the strontium initial ratio (Fig. 1). Besides the uncertainties introduced by the analytical determination of ^{87}Sr/^{86}Sr and ^{87}Rb/^{86}Sr, the only source of error is that of the decay constant of the parent nuclide ^{87}Rb. Because its decay energy is very low, its direct determination is relatively unprecise. The current value is λ^{87}Rb $1.42\pm0.01\times10^{-11}$ yr^{-1} (refs 1, 2). Another way to obtain this constant is to compare ages obtained by the ^{87}Rb–^{87}Sr method with those obtained by the U–Pb method[1,3]. Indeed, as the decay constant of ^{235}U is much larger than that of ^{87}Rb, it is easier to measure and its uncertainty is $<0.3\%$ (ref. 4). We now compare ^{87}Rb–^{87}Sr and U–Th–Pb ages for chondrites and indicate that, in order for the ages to agree, the ^{87}Rb decay constant would have to be changed.

Compared with dating terrestrial or lunar samples, dating of meteorites presents a major difficulty: because their origin is uncertain, it is impossible to know in advance whether they are genetically related. The best approach is therefore to analyse a set of meteorites and, if an isochron is obtained, to infer that they are genetically related, or at least formed within a short interval of time compared with the uncertainty of the dating. In the early works on ^{87}Rb–^{87}Sr dating of meteorites, objects of widely varying compositions, including chondrites and basaltic achondrites, were analysed[5–9]. As the latter are differentiated objects, largely deficient in Rb compared with chondrites, fairly well-defined 'isochrons' were obtained which gave an age of 4,560 Myr, with an error limit of the order of 150 Myr.

Clearly, however, such drastically different objects cannot be genetically related in a simple way. A 'primitive' ^{87}Rb–^{87}Sr isochron should use only chondrites. Moreover, chondrites can be classified into chemical groups[10]. Oxygen isotopes studies have recently provided some evidence in favour of this classification: indeed, the chondrites from a given group tend to have similar oxygen isotopic composition[11]. Though this result is not systematic, it indicates that the chondrites from a given group originated from a single reservoir of the solar nebula. It is thus preferable to date chondrites group by group. The first attempt of such an analysis[12] has been followed by a systematic study by Wetherill and his co-workers[13,14]. This resulted in a set of isochrons for carbonaceous, ordinary and enstatite chondrites which were grouped by Wetherill[15] into a

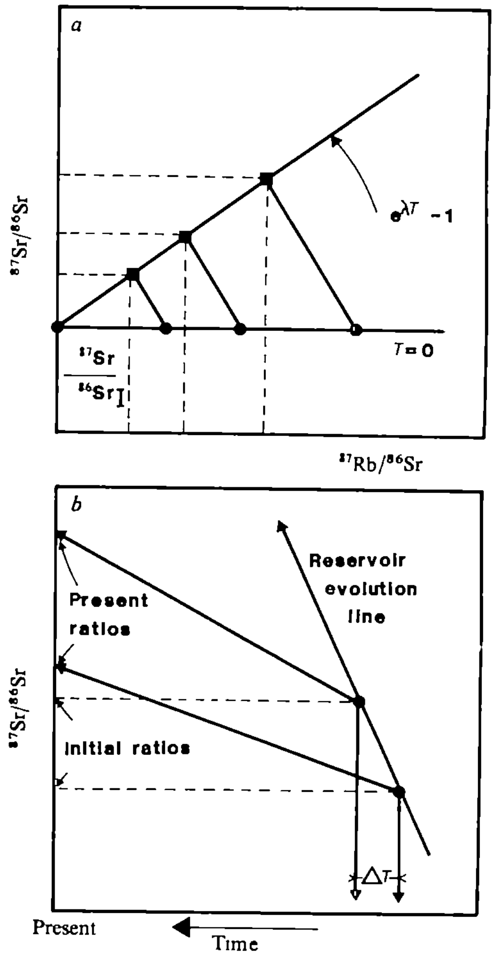

Fig. 1 *a*, Schematic evolution of the ^{87}Sr/^{86}Sr isotopic compositions of a series of objects formed simultaneously with different Rb/Sr ratios. Such objects plot on a straight line called isochron whose slope yields the 'age' of the objects. *b*, Evolution with time of the ^{87}Sr/^{86}Sr ratio in a reservoir. The higher the Rb/Sr ratio in the reservoir, the faster this evolution. The difference between the initial ratio of two objects formed from a single reservoir can be used to calculate an age difference.

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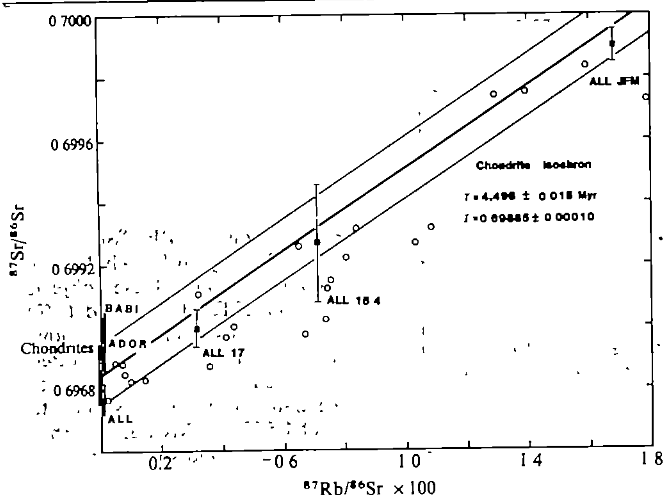

Fig. 2 Plots of $^{87}Rb/^{86}Sr$ against $^{87}Sr/^{86}Sr$ for some Allende refractory inclusions. ■, New values (see ref. 45): ALL 17 – $^{87}Rb/^{86}Sr = 0.00316$ – $^{87}Sr/^{86}Sr = 0.69900 \pm 0.0006$; ALL 15.4 – $^{87}Rb/^{86}Sr = 0.00714$ – $^{87}Sr/^{86}Sr = 0.69927 \pm 0.00014$; ALL JFM – $^{87}Rb/^{86}Sr = 0.0168$ – $^{87}Sr/^{86}Sr = 0.69990 \pm 0.00006$. ○, Data from the literature (refs 19–23). Since Allende refractory inclusions have been disturbed, extrapolation from the actual data points to an initial ratio can be meaningless. The Allende initial ratio (ALL) is obtained from a measured value. The $^{87}Sr/^{86}Sr$ initial values BABI[17,18] and ADOR[41] are indicated as well the error envelope of the isochron from Fig. 4.

single isochron of age (4,520±40) Myr and strontium initial ratio 0.7003±0.0004.

At the same time, Wasserburg and his co-workers demonstrated that it was possible to measure the isotopic composition of strontium at a precision of better than 10^{-4} (ref. 16) and obtained a very well-defined strontium initial ratio for basaltic achondrites of $^{87}Sr/^{86}Sr_{BABI} = 0.69899 \pm 0.00005$ (ref. 17, see also ref. 18). They have also proposed the use of the strontium initial ratio as a cosmochronometer. The principle of this technique is (Fig. 1): due to the decay of ^{87}Rb, the $^{87}Sr/^{86}Sr$ isotopic ratio increases with time in any reservoir. The larger the Rb/Sr ratio of the reservoir, the faster this increase. The difference between the strontium initial ratio of two objects formed from a same reservoir gives the time elapsed between their formation, provided the Rb/Sr ratio of the reservoir is known. Basaltic achondrites are differentiated, that is, have suffered magmatic events after their formation; thus it could be expected that their initial ratio should be higher than that for chondrites. Indeed, that ratio for the refractory inclusions of the carbonaceous chondrite Allende was found to be $^{87}Sr/^{86}Sr_{ALL} = 0.69877 \pm 0.00005$ (refs 19–21; also refs 22, 23 and Fig. 2). Yet the chondrite isochron of Wetherill[15], some precise data for low-radiogenic chondrites[20] as well as an internal isochron for the chondrite Guareña[24] indicated strontium initial ratios higher than BABI. Clearly more data with an improved accuracy were needed.

Since chondrites are radiogenic (the mean $^{87}Sr/^{86}Sr$ ratio for chondrites is about 0.75), the error on chondrite isochrons is dominated by that on the abscissa $^{87}Rb/^{86}Sr$. We developed a technique for measuring the rubidium isotopic composition with a reproductibility of better than 0.2% allowing a precision of ±0.5% for the $^{87}Rb/^{86}Sr$ ratio[25]. This represented an increase of the precision for this ratio by a factor of 2–4 compared with the previous studies; joined with a better than 10^{-4} precision for the strontium isotopic composition[18], this allowed a re-analysis of the problem. Specifically, we wanted to answer the following questions:

(1) Do chondrites still define ^{87}Rb–^{87}Sr isochrons with this increased precision?

(2) Are there differences between the different groups of chondrites?

(3) What is the strontium initial ratio for chondrites?

(4) What is the ^{87}Rb–^{87}Sr age for chondrites?

The results for the whole rock and mineral studies of the different groups of ordinary and enstatite chondrites have been published recently[25–27]. However, the joint results of these group studies as well as their discussion have not yet been presented and are given here, with a new set of analyses for Allende white inclusions, carried out in our laboratory. Most of the recent results from other laboratories on ^{87}Rb–^{87}Sr dating of chondrites will be incorporated into this discussion.

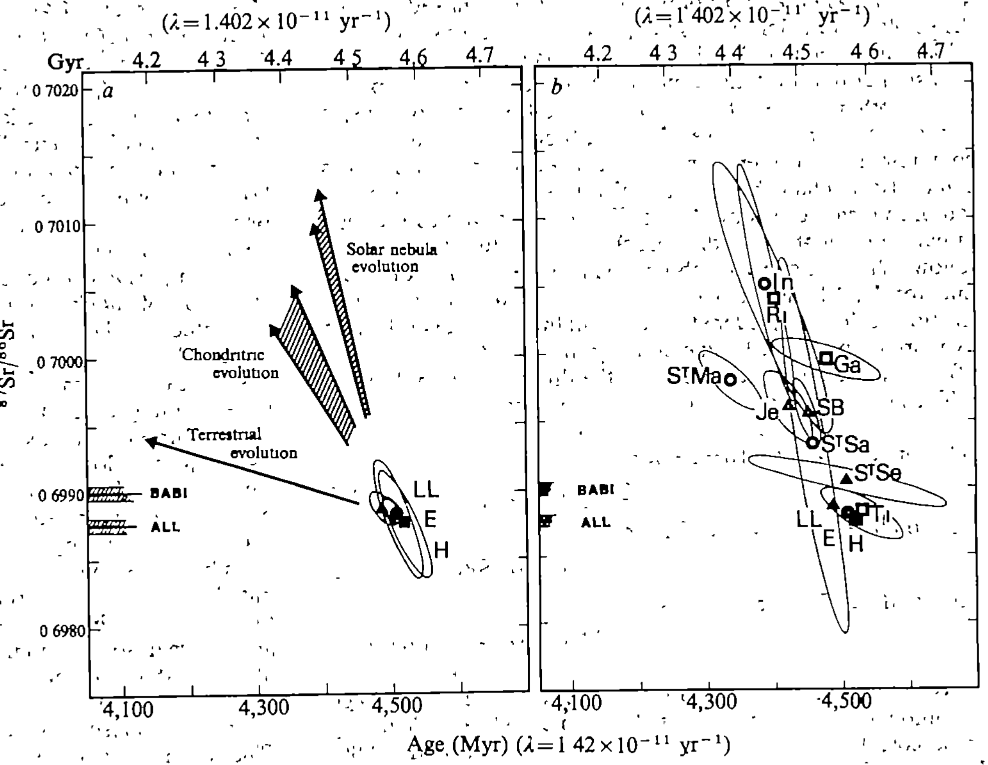

Fig. 3 *a*, Plot of age against strontium initial ratio for the chondrite whole-rock isochrons. The ellipse representation is justified in ref. 26. The smaller ellipse corresponds to the 'chondrite isochron' of Fig. 4. The strontium initial ratios ALL and BABI are also shown. The arrows indicate the direction of evolution of the $^{87}Sr/^{86}Sr$ ratio with time in reservoirs of solar (Rb/Sr = 0.5 to 0.6), chondritic (Rb/Sr = 0.2 to 0.3) and terrestrial (Rb/Sr = 0.04) composition. *b*, Age against strontium initial ratio for internal isochrons of chondrites. Ti, Tieschitz (H3 – 4,530±60 Myr; $I = 0.69880 \pm 0.00024$)[25]; Ri, Richardton (H5 – 4,400±90 Myr; $I = 0.7004 \pm 0.0010$)[30]; Ga, Guareña (H6 – 4,480±80 Myr; $I = 0.69995 \pm 0.00015$)[24]; SB, Soko Banja (LL4 – 4,420±20 Myr; $I = 0.69954 \pm 0.00024$)[25]; St Se, Saint Severin (LL6 – 4,510±150 Myr; $I = 0.69903 \pm 0.00020$)[27]; Je, Jelica (LL6 – 4,423±41 Myr; $I = 0.69959 \pm 0.00029$)[25]; In, Indarch (E4 – 4,393±43 Myr; $I = 0.7005 \pm 0.0009$)[26]; St Ma, Saints Marks (E5 – 4,335±50 Myr; $I = 0.69979 \pm 0.0022$)[26]; St Sa, Saint Sauveur (E5 – 4,457+47 Myr; $I = 0.6993 \pm 0.0014$)[26]. Within error limits, these isochrons can be interpreted as closed system internal isotopic re-equilibration of the meteorites.

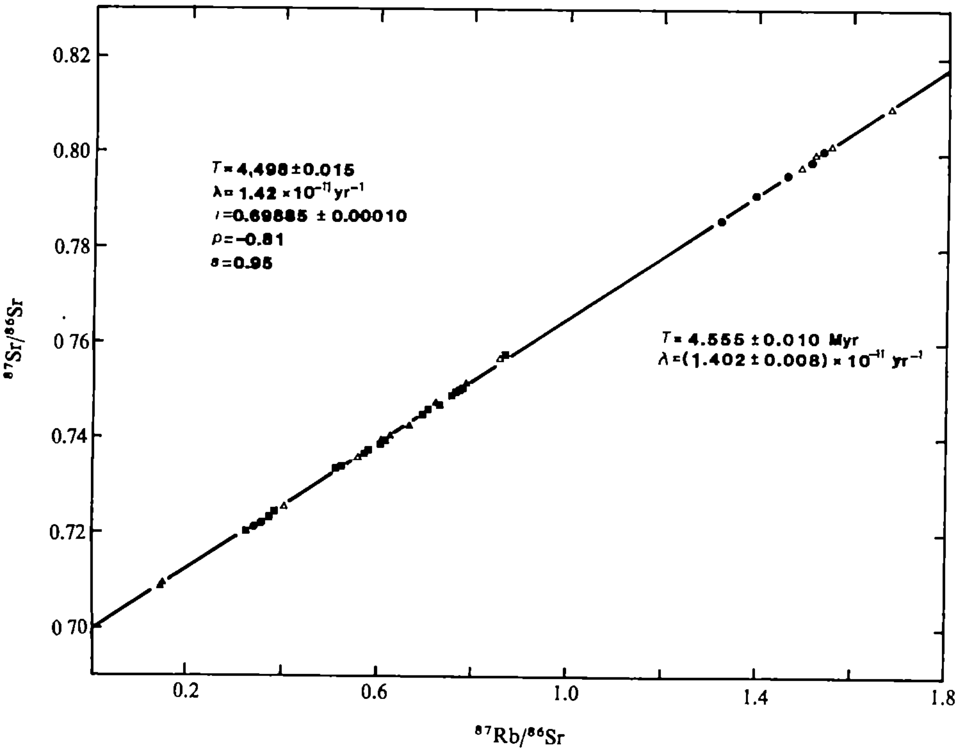

Fig. 4 Plots of $^{87}Rb/^{86}Sr$ against $^{87}Sr/^{86}Sr$ diagram for whole rock chondrites. Only these objects from the H(■), LL(△) and E(●) chondrites used in the previous isochron calculations are plotted and used in the least-squares regression. If the age is assumed to be of 4,555±10 Myr, then $^{87}Rb = 1.402 \pm 0.008 \times 10^{-11}\ yr^{-1}$.

Comparative chronology of chondrites

(1) Do chondrites still define whole-rock isochrons when the precision of the measurements is increased?

The answer to this question is positive: with a few exceptions, H, LL, and E-type chondrites define very precise isochrons. On the other hand, L chondrites scatter in the $^{87}Rb/^{86}Sr$ against $^{87}Sr/^{86}Sr$ diagram[25]. Our preferred interpretation is that Rb has been lost by volatilization from these meteorites during the severe shocks which affected most of them: those which are unshocked tend to plot close to a reference isochron of age 4,500 Myr, whereas most of the shocked and degassed L chondrites plot on the left of this line, possibly indicating a lowered Rb/Sr ratio compared with their initial one.

A similar interpretation could explain most of the exceptions for the other groups of chondrites: for all of these objects, it is possible to find evidence of shock, brecciation or terrestrial contamination or alteration[25–27]. Such secondary disturbances could also explain the high initial ratios found for some low radiogenic chondrites by Wetherill *et al.*[20], as well as the high initial ratio for the chondrite isochron of Wetherill[15]. In what follows these 'disturbed' objects will be rejected. Note here that for H, LL and E chondrites, these amount to a small fraction of the analysed meteorites, and that they generally plot by more than 3σ off the line defining the others.

(2) Are there differences between the different groups?

The ages for the other groups were respectively:

H chondrites $T = 4{,}518 \pm 39$ Myr:

$$^{87}Sr/^{86}Sr_I = 0.69876 \pm 0.00040$$

LL chondrites $T = 4{,}486 \pm 20$ Myr:

$$^{87}Sr/^{86}Sr_I = 0.69887 \pm 0.00012$$

E chondrites $T = 4{,}508 \pm 37$ Myr:

$$^{87}Sr/^{86}Sr_I = 0.69880 \pm 0.00044$$

As noted by Sanz and Wasserburg[28], the errors on the age and initial ratio are anticorrelated. Our method[26] of calculating these correlation coefficients, gives −0.96, −0.73 and −0.80. We have also shown that, in a diagram where one plots the initial ratio against the age, an ellipse is always a reasonable representation of the error limits. The error ellipses for the above isochrons are represented in Fig. 3. They are clearly undistinguishable from each other; in other words, the ^{87}Rb–^{87}Sr age of all chondrites is identical within error limits.

(3) What are the internal isochrons of chondrites with respect to those for whole rocks?

It might be argued that to be sure that the different analysed objects be cogenetic, the best method is to analyse separate mineral phases of a given meteorite. Quite a few ^{87}Rb–^{87}Sr internal studies of chondrites have now been achieved. These are Allende (CV)[19–23] (and this work), Tieschitz (H3)[25], Lost City (H5)[29], Richardton (H5)[30], Guareña (H6)[24], Mező Madaras (L3)[25], Bjürbole (L4)[31], Orvinio (L6)[13], Farmington (L6)[13], Bruderheim (L6)[32], Chainpur (LL3)[25], Soko Banja (LL4)[25], Olivenza (LL5)[28], Guidder (LL5)[25], Krähenberg (LL5)[33], Peace River (LL6)[32,19], Saint Séverin (LL6)[27], Saint Mesmin (LL5)[25], Ensisheim (LL6)[25], Jelica (LL6)[25], Abee (E4)[32,26], Indarch (E4)[13,26], Saint Sauveur (E5)[26], Saint Mark's (E5)[26].

For some, the data obviously present analytical difficulties. Some do not have the necessary precision, number of analyses or range of the values in the ^{87}Rb–^{86}Sr, ^{87}Sr–^{86}Sr diagram, to be useful for comparative chronology. In addition, most of the remaining results do not define an isochron. In most cases, this can result of shock resetting or terrestrial alteration. We have presented in Fig. 3 the few which we consider reliable and useful. The obvious result is that only the H3 chondrite Tieschitz has the same internal age as that for whole rocks. All others have a younger age and a higher $^{87}Sr/^{86}Sr$ initial ratio. Note that within error limits, all these internal isochrons are consistent with a closed system reequilibration of the meteorites. Because Tieschitz is an unequilibrated object, whereas most others are equilibrated, this is interpreted as being due to the cooling or metamorphism history of the latter[25–27]. As for E-chondrite, this could reveal shock effects[26]. From the $^{87}Sr/^{86}Sr$ initial ratio of these internal isochrons, it is possible to estimate the interval of time elapsed between the chondrite formation and internal isotopic homogenization[17]. For the type 6 objects of Fig. 3, this interval is of the order of 100 Myr. It is shorter, of the order of 40 Myr, for the type 4 object Soko Banja.

The internal isochron for Tieschitz is a 'chondrule isochron'. This means that it has not been obtained by analysing pure mineral phases, but whole chondrules[25]. This result shows that, at the precision limits of the method, chondrules were formed simultaneously with whole rock chondrites.

The whole rock best isochron for chondrites

Comparison of the ages of the various chondrite classes shows that they are undistinguishable within error limits. It is thus justified to use all data together and determine a $^{87}Rb-^{87}Sr$ 'best' age for chondrites. The results are shown in Figs 4 and 5: the best age for chondrites is 4,498±15 Myr for $\lambda\ ^{87}Rb = 1.42\times10^{-11}\ yr^{-1}$.

Let us consider the consequences of this result.

The precision for this age is the 'best' ever obtained for a $^{87}Rb-^{87}Sr$ isochron; it is comparable to that for extinct radioactivities such as $^{129}I-^{129}Xe$ (see ref. 34), but relates to an absolute age. Since the scatter of the data points around the isochron is small (the reduced X^2 is 0.95), this error limit is probably significant. On Fig. 5, we have represented the $^{87}Rb-^{87}Sr$ model age of the various chondrites relative to the $^{87}Sr/^{86}Sr$ initial ratio of the isochron from Fig. 4. Most results indeed agree with the best fit line within error limits: 95% of the data points plot within an interval of ±50 Myr. More than 80% plot within an interval of ±30 Myr. This result confirms the idea of a 'sharp isochronism' of formation of chondrites, on a time scale of a few million years as deduced from I–Xe chronology[34].

The question is then to relate this age to the processes of formation of the Solar System. The isochron is based on variations of the Rb/Sr ratio among chondrites. As the Sr concentration for the objects of a given group is constant[35] (Fig. 6), the variations are due to variations of the Rb concentration. It is generally believed that the latter is either the result of fractionation of moderately volatile elements during condensation–accretion, or of volatilization and loss during the reheating and metamorphic events which affected most chondrites (see ref. 36). $^{87}Rb-^{87}Sr$ dating does not help to decipher which process is being dated. However, in order not to perturb the whole rock isochron, the latter process should have occurred during the first few million years of the chondrites. In particular it is unlikely that Rb was lost some 100 Myr after the formation of these meteorites, at the time dated by internal $^{87}Rb-^{87}Sr$ isochrons of equilibrated chondrites. Rb is also fractionated among various clasts of a given chondrite and it is not generally known when such clasts assembled. If it could be demonstrated that their assembly occurred before metamorphism, this would be a strong evidence in favour of Rb fractionation during condensation–accretion.

It is known that Rb is highly fractionated in the Earth compared with ordinary chondrites: from the Sr–Nd isotopic correlation in terrestrial basalts, it is estimated that the 'bulk Earth' $^{87}Rb/^{86}Sr$ ratio is 0.098 (refs 37, 38). Not that only Ensisheim presents such a low value. It is not certain whether the fractionation processes which affected moderately volatile elements were the same inside the chondrite parent bodies as they were in a whole terrestrial planet. Yet the present results indicate that fractionation could attain the same efficiency on very small to very large distance scales. Identification of these processes could result in very strong constraints for the chronology of formation of the Earth.

The best age for chondrites is different from the most recent $^{206}Pb-^{207}Pb$ ages for meteorites. The latter can be very precise because they rely on isotopic ratios only. However, their actual values are very sensitive to small disturbances[39]. If one selects the data for which the U–Pb or U–Th–Pb ages are concordant, which means that the ages deduced from the independent chronometers $^{235}U-^{207}Pb$, $^{238}U-^{206}Pb$ and $^{232}Th-^{208}Pb$ are equal, the corresponding $^{206}Pb-^{207}Pb$ ages fall around 4,555±10 Myr, whether for whole rocks chondrites[39], for chondritic minerals[27], or for achondrites[41]. We do not intend to discuss the problems of U–Pb dating of chondrites fully here but it is possible that the actual U–Pb age of some chondrites is as old as 4,580 Myr (ref. 39). In any case, even the 4,555 Myr value is 1% older than our $^{87}Rb-^{87}Sr$ age. It seems unrealistic that such a difference is real, and that Pb–Sr and U–Th–Pb fractionations occurred independently on a time scale of about 50 Myr.

As all our measurements were made using the same method, and the same spike solution[25–27], such a difference could be due to a systematic bias in our technique. The main possible source for such a bias would be the spike calibration. However, our spike has been repetitively calibrated against different normal solutions and the results agreed within 0.2%. In addition, these spike solutions were used to analyse the Rb and Sr concentrations in NBS glass wafers and our results agreed within 0.2% of the certified values. Finally, our values agree very well with those obtained by others[30].

On the whole, we believe that a 1% bias in our measurements is unreasonably large. A possible alternative is that the ^{87}Rb decay constant is still incorrectly estimated. Our results compared with the $^{206}Pb-^{207}Pb$ ages would indicate that $\lambda^{87}Rb = (1.402\pm0.008)\times10^{-11}\ yr^{-1}$. Compared with the value of $1.42\times10^{-11}\ yr^{-1}$, this would change $^{87}Rb-^{87}Sr$ ages by a factor of 1.013. This should be verified by comparison of other simultaneous Rb–Sr, Sm–Nd and Pb–Pb measurements; however, such studies should attain a better than 1% precision for eventual differences to be significant.

Note that K–Ar ages of chondites are also younger than U–Th–Pb ages: Turner *et al.*[40] found an average $^{39}Ar-^{40}Ar$ plateau age of 4,480±30 Myr for such objects, the older one being of 4,520 Myr. Their interpretation is that the K–Ar system of the bulk meteorites has been reset during metamorphism on a time scale of 100 Myr. If such was the case, one would expect that Rb should also be redistributed simultaneously during this process but, as discussed above, this seems difficult. In addition, no systematics of $^{39}Ar-^{40}Ar$ ages against the degree of metamorphism is found. Alternatively, a bias in the ^{40}K decay constant joined by larger than estimated error limits would render the problem simpler to understand. Again, these questions warrant more data (see the discussion in ref. 25).

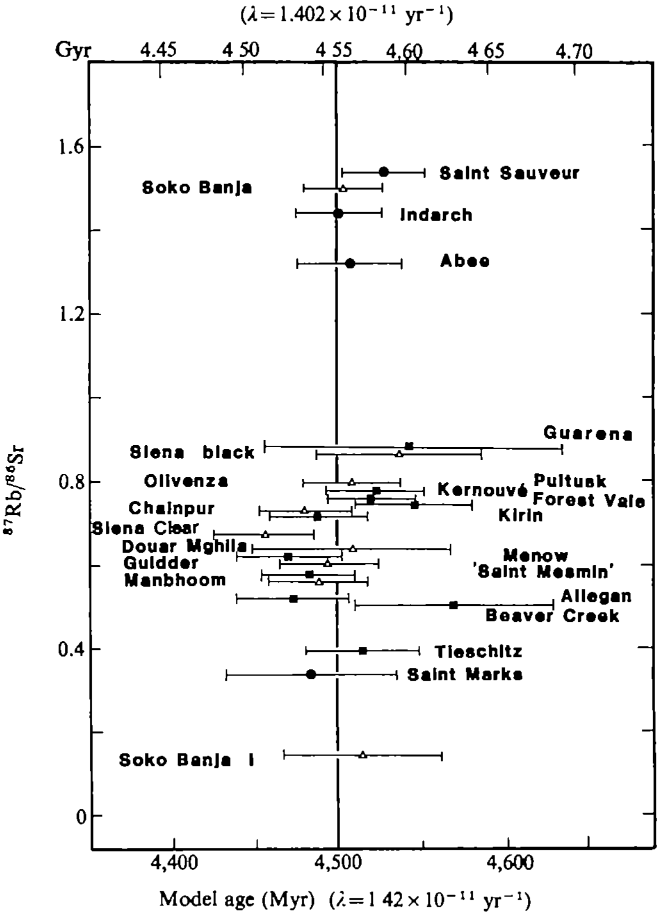

Fig. 5 Model $^{87}Rb-^{87}Sr$ ages of chondrites. The $^{87}Sr/^{86}Sr$ initial ratio is assumed to be that of our best fit isochron (Fig. 4). The different analyses for a given object have been averaged. All ordinary and enstatite chondrites have undistinguishable ages.

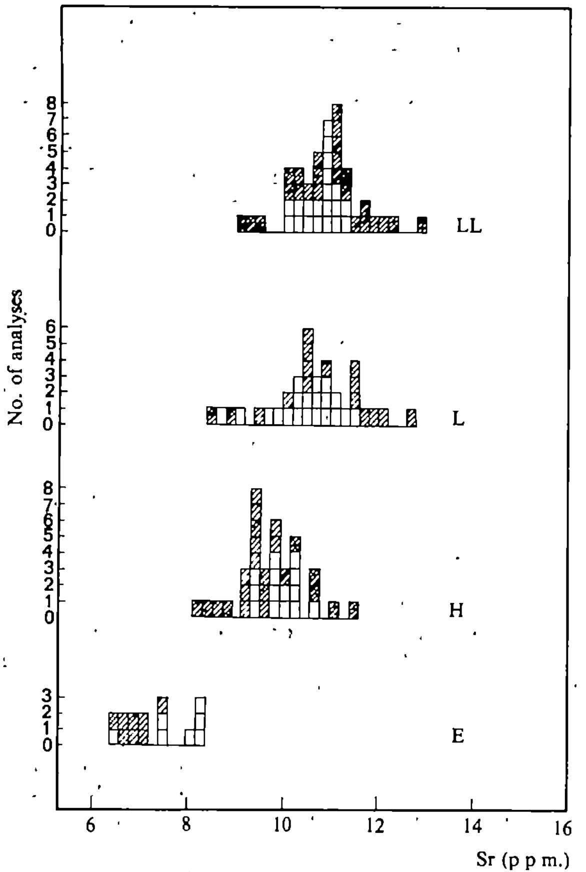

Fig. 6 Sr concentration in ordinary and enstatite chondrites. Our data[25,26] (▨) and these of Gopalan and Wetherill[35] (☐) are included. Only objects which plot close to the Rb–Sr isochron have been considered. Much of the scatter is probably due to the non-representativity of the samples. The Sr concentration within each group is constant.

The $^{87}Sr/^{86}Sr$ initial ratio for chondrites is 0.69885 ± 0.00010. Figures 2 and 3 also show $^{87}Sr/^{86}Sr_{BABI}$ and $^{87}Sr/^{86}Sr_{ALL}$, where $^{87}Sr/^{86}Sr_{BABI}$ is the initial ratio for basaltic achondrites[17], and $^{87}Sr/^{86}Sr_{ALL}$ is that for Allende white inclusions[19]. The value for basaltic achondrites has been confirmed in our laboratory[18]. We also show in Fig. 2 a few data on Allende white inclusions. We assume that no interlaboratory bias has been introduced in the strontium initial ratios for meteorites, and that for the 'chondrite isochron' is significantly lower than BABI and undistinguishable from ALL. Thus, the expected result that the differentiated achondrites have a strontium initial ratio higher than that of chondrites is confirmed.

Yet, from Figs 2 and 3, it might appear that ALL is slightly lower than the chondrite initial ratio. The actual value for chondrites is very close to that observed in the differentiated meteorite Angra Dos Reis (Ador, see for example ref. 41). Such a difference could be due to a time delay in the chondritic fractionation of Rb relative to the formation of the Allende refractory inclusions. Assuming that both formed directly from the solar nebula, the Rb/Sr ratio of which is 0.6 to 0.5 (ref. 42), the time delay would be 3.0 ± 5.6 Myr to 3.9 ± 7.3 Myr. If the Rb fractionation which is being dated by the chondrite isochrons occurred during chondrite metamorphism, these objects would have evolved in a reservoir of chondritic composition (Rb/Sr = 0.3 to 0.2) and the time delay would be 7.0 ± 13.2 to 9.4 ± 17.6 Myr. Thus, chondrites and Allende refractory inclusions are 'synchronous' on a time scale of 10 Myr. This time scale is comparable with that deduced from I–Xe chronology[34]. Unfortunately, it is not precise enough for constraining the models of formation of planetesimals (see ref. 43), since all these models deal with time scales shorter than 10 Myr.

Another indirect result of the existence of an isochron is that there was no large anomaly for the ^{87}Sr and ^{86}Sr isotopic abundances at the time of formation of chondrites in the areas of the solar nebula where these meteorites formed. Evidence has been found for isotopic abundance anomalies of ^{84}Sr in some rare refractory inclusions of the Allende carbonaceous chondrite[44]. It is not clear whether there were some anomalies for ^{87}Sr and ^{86}Sr in this material. For whole rock chondrites, the present evidence is that isotopic anomalies for these isotopes were small enough for the ^{87}Rb–^{87}Sr dating technique to be fully useable.

Conclusions

The major conclusion of our study is that all chondrites formed simultaneously from a solar nebula where the $^{87}Sr/^{86}Sr$ ratio was homogeneous; the $^{87}Sr/^{86}Sr$ initial ratio for chondrites is undistinguishable from that for carbonaceous chondrites.

This ratio is significantly lower than BABI, the initial ratio for basaltic achondrites[17,18]. This can be interpreted as a later differentiation of the parent body of these latter objects. However, the matter is complicated by the fact that there exists at least one differentiated object with an initial ratio lower than BABI and undistinguishable from that for chondrites (ADOR, ref. 41). In addition, ^{87}Rb–^{87}Sr dating does not give any information about the predifferentiation history of these bodies (see ref. 18). The same applies for the Earth and the Moon. Yet, our results indicate that use of the ALL value of 0.69877 ± 0.00005 (ref. 19) as a starting value for the history of all these planets is not unplausible.

Using the ^{87}Rb decay constant of $\lambda = 1.42 \times 10^{-11}$ yr^{-1}, the ^{87}Rb–^{87}Sr age for chondrites is 4,498 ± 15 Myr. For the Rb–Sr and U–Th–Pb chronometers to agree with each other at 4,555 Myr, this decay constant would be $1.402 \pm 0.008 \times 10^{-11}$ yr^{-1}. This result calls for further simultaneous precise Rb–Sr, Sm–Nd and U–Th–Pb analyses of selected samples. It should certainly be considered in the comparison of precise ages obtained by different chronometers.

^{87}Rb–^{87}Sr dating of chondrites relates to Rb fractionation in the early Solar System. This occurred either during condensation–accretion or at an early stage of metamorphism of chondrites. The ^{87}Rb–^{87}Sr internal system has been perturbed in most chondrites, probably due to shock and thermal metamorphism: type 3 unequilibrated chondrites are an exception and can be considered unique.

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Human chorionic gonadotropin β-subunit is encoded by at least eight genes arranged in tandem and inverted pairs

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The β-subunit of the human placental glycoprotein hormone, chorionic gonadotropin (HCG), is coded for by at least eight different genes or pseudogenes, four of which are arranged in inverted pairs while the other four are arranged in tandem pairs. The genes are each 1.45 kilobases (kb) long and have two short introns located in identical positions.

THE placental hormone, human chorionic gonadotropin (HCG), together with the three pituitary hormones, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH), comprise the glycoprotein hormone family. All four hormones are dimeric with two different subunits, α and β, which are associated noncovalently (for review see ref. 1).

Amino acid sequence analysis has shown that the α-subunit seems to be common to the four hormones, while the β-subunits are unique and confer biological specificity on the individual hormones. Varying degrees of amino acid sequence homology exist between the four β-subunits, which led to the proposal that they evolved from a common ancestral gene[1].

We have previously isolated and characterized cDNA clones encoding the α-(ref. 2) and β-(ref. 3) subunits of the placental hormone, HCG. The α-subunit cDNA was then used as a hybridization probe to purify the corresponding genomic fragment[4]. Comparison of the structure of the cloned α-subunit gene with the hybridizing restriction enzyme fragments in human DNA led to the conclusion that there is only a single α-subunit gene which is transcribed to make the α-subunit mRNA for all four hormones[4]. A similar conclusion was also reached by Boothby *et al.*[5], who used an α-subunit cDNA to probe uncloned human DNA.

Here we show that the gene structure for the β-subunit of HCG is much more complex than that of the single α-subunit gene. At least eight distinct β-HCG or β-HCG-like genes have been identified by a combination of filter hybridization of genomic DNA and isolation of bacteriophage λ genomic recombinants. These genes, which are structurally very similar, form a family of sequences which are probably all linked.

Isolation of β-HCG genomic recombinants

The β-HCG cDNA was used to isolate genomic recombinants from two independent human chromosomal libraries, one in bacteriophage λ Charon 4A (ref. 6) and one in bacteriophage λ Charon 28 (E. Fritsch and C. Sabourin, unpublished results). We purified 16 hybridizing phage, 9 from the Charon 4A library and 7 from the Charon 28 library. Restriction enzyme maps for these phage DNAs were determined by analysing a combination of single and double restriction enzyme digests using the filter hybridization method of Southern[7]. We thus identified three different groups of overlapping recombinants (the restriction enzyme maps for these three groups, *a*, *b* and *c*, are shown in Fig. 1).

β-HCG genes are arranged in both inverted and tandem pairs

Eight separate β-HCG, or β-HCG-like, genes or pseudogenes have been identified by hybridization in the three groups, *a*, *b*, and *c*; the location and polarity of these genes, termed β-HCG 1–8, are shown in Fig. 1.

Four of the genes, β-HCG 1 and 2 and β-HCG 5 and 6, are found in inverted pairs. A detailed map of the region containing the gene pair β-HCG 5 and 6 is shown in Fig. 2; DNA sequencing of the subcloned fragments identified in this figure confirmed the inverted orientation of the genes. Similarities between the restriction enzyme patterns of these β-HCG 5 and 6 subclones with similar subclones containing β-HCG 1 and 2 showed that these two genes also form an inverted pair.

In contrast to these four genes, β-HCG 3 and 4 and β-HCG 7 and 8 form tandem gene pairs. We have shown this again by comparing restriction enzyme digestion patterns of subcloned fragments and, in the case of β-HCG 3, by DNA sequencing.

β-HCG genes contain two introns in identical positions

DNA sequencing of four of the eight β-HCG genes (β-HCG 2, 3, 5 and 6; K. Talmadge, N.C.V., W.R.B. and J.C.F., unpublished results) reveals that they all have two introns that separate codon -16 of the signal peptide from codon -15, and codon 41 of the mature protein from codon 42. The two introns,

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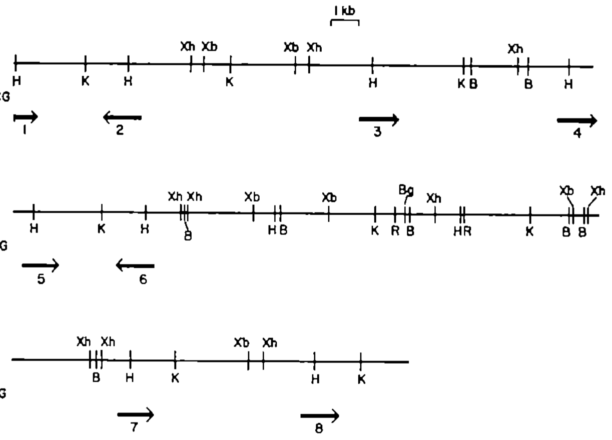

Fig. 1 Restriction enzyme maps of the three goups of β-HCG genomic isolates (*a*, *b* and *c*). H = *Hin*dIII, K = *Kpn*I, Xh = *Xho*I, Xb = *Xba*I, B = *Bam*HI, Bg = *Bgl*II and R = *Eco*RI. The map for group *a* (21.5 kb long) was constructed from 10 overlapping phage isolates, that for group *b* (22.0 kb) from 5 isolates and that for *c* (14.7 kb) from a single isolate. The positions of the eight β-HCG genes (1–8) are indicated by arrows which point in the proposed direction of transcription. Each gene spans ~1.45 kb from the cap site to the poly(A)site. These phage isolates were obtained either from a genomic library made from human fetal liver DNA, partially digested with *Hae*III and *Alu*I, and cloned with *Eco*RI linkers into bacteriophage λ Charon 4A (a gift from T. Maniatis[6]) or from a second library, made from DNA isolated from the chemically transformed human fibroblast cell line, HAI, and cloned as an *Mbo*I partial digest in the vector bacteriophage λ Charon 28 (a gift from E. Fritsch). These libraries, and the individual bacteriophage isolates, were propagated in *E. coli* K803 in P1 conditions in accordance wth the NIH Guidelines for Recombinant DNA Research. The 579-base pair (bp) *Hin*dIII fragment containing the cloned β-HCG cDNA isolated previously[3] was ^{32}P-labelled either by nick-translation[12] or by priming with calf thymus DNA oligonucleotides[13]. Recombinant phage plaques were screened on Schleicher and Schuell 0.45-μm nitrocellulose filters by the method of Benton and Davis[14] as described elsewhere[4]. Restriction endonucleases were from New England Biolabs. Digests of DNA isolated from recombinant bacteriophage were analysed by electrophoresis on 1.0% agarose gels, followed by transfer to nitrocellulose filters and hybridization by the method of Southern[7] as described elsewhere[4]. The sizes of fragments less than 2 kb long were estimated on 3% acrylamide gels.

named A and B, are ~350 and 230 nucleotides long, respectively. Figure 3 shows a composite structure for these genes. As the other genes, β-HCG 1, 4, 7 and 8, have restriction enzyme patterns very similar to the partially sequenced β-HCG genes, we expect them also to have two introns at the same positions.

The *Hin*dIII site in intron A (Fig. 3) is the *Hin*dIII site at the end of the 2.6- and 1.6-kb *Hin*dIII-*Kpn*I fragments identified in Fig. 2. These fragments, which contain β-HCG 5 and 6 respectively, were subcloned into plasmid vectors. The 5′ exon and the promoter region for β-HCG 5 and 6 are contained on a 0.8-kb *Hin*dIII to *Eco*RI fragment and a 1.4-kb *Hin*dIII to *Bam*HI fragment, respectively (Fig. 2). These fragments were also isolated from a phage λ recombinant and subcloned into plasmids. The DNA sequencing used to generate the composite map in Fig. 3 was obtained from these subclones.

The 0.8-kb *Hin*dIII to *Eco*RI fragment of Fig. 2 was also used as a hybridization probe in filter hybridization analysis of all the phage λ clones. This fragment, which contains the 5′ end of β-HCG 5, hybridizes strongly to the region upstream from the *Hin*dIII site observed in all the β-HCG genes. This information, together with the detailed restriction enzyme analysis of the region upstream from these *Hin*dIII sites, further supports our conclusion that all eight β-HCG genes have the same general structure with two intervening sequences in identical positions. Small differences between the genes, and the possibility that there may be pseudogenes, cannot be excluded until the DNA sequence analysis is completed.

Correlation of cloned fragments with those in total genomic DNA

To determine whether the cloned β-HCG genes correlate with the hybridizing fragments seen in total genomic DNA and to estimate the number of β-HCG hybridizing genes or pseudogenes, we digested human placental DNA with several restriction endonucleases, followed by filter hybridization analysis[7]. Two separate probes were used: the β-HCG cDNA specific for the region to the 3′ side of the *Hin*dIII site located in intron A (Fig. 3), and the 0.8-kb *Hin*dIII to *Eco*RI fragment specific for the region to the 5′ side of this site (Fig. 2). An autoradiograph of a filter hybridization of genomic DNA with the 3′ probe is shown in Fig. 4 while one with the 5′ probe is shown in Fig. 5. Both of these probes identify a fairly complex pattern of hybridizing fragments. This is consistent with the conclusion, drawn from the analysis of the phage λ recombinants, that there are multiple regions hybridizing to the β-HCG cDNA. The intensity differences among the hybridizing bands make it difficult to estimate accurately the number of β-HCG sequences.

Examination of the restriction enzyme maps of Fig. 1 suggests that in a *Kpn*I digest of genomic DNA, each gene should be contained in a unique fragment that is recognized by both the 5′ and the 3′ probes. Similarly, in a combined *Hin*dIII-*Kpn*I digest, the genes should be separated into two parts that are recognized exclusively by either probe. The hybridization patterns observed in the filter hybridizations of human genomic DNA are consistent with these expectations. With *Kpn*I alone, both probes (Figs 4, 5) identify the same set of bands, but, as a result of intensity differences, it is hard to estimate the number of genes represented by these bands. With the combined *Hin*dIII–*Kpn*I digests, both probes (Figs 4, 5) give different, but rather simpler, patterns. For example, in the case of the 3′ probe with a *Hin*dIII–*Kpn*I digest (Fig. 4), four bands of 3.5, 2.6, 2.2 and 1.6 kb are identified. Examination of restriction enzyme maps of the cloned genes (Fig. 1) reveals that β-HCG genes 1 and 5 are both on 2.6-kb *Hin*dIII–*Kpn*I fragments, β-HCG 2, 6, 7 and 8 are on 1.6-kb fragments, β-HCG 3 is on

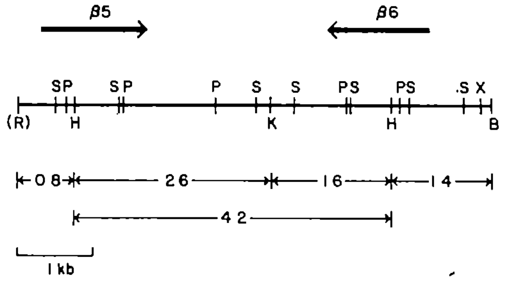

Fig. 2 Detailed restriction enzyme map for the region of group *b* containing the symmetrical pair of genes β5 and β6. The polarity of these genes is indicated by arrows. Restriction enzyme sites are shown for *Eco*RI (R), *Hin*dIII (H), *Kpn*I (K), *Bam*HI (B), *Sac*I (S), *Xho*I (X) and *Pst*I (P). The *Eco*RI site shown in parentheses is the result of linker ligation in the construction of the Charon 4A library. This map was obtained from an analysis of the following subcloned fragments whose positions are indicated: *Eco*RI–*Hin*dIII, 0.8 kb; *Hin*dIII–*Kpn*I, 1.6 and 2.6 kb; *Bam*HI–*Hin*dIII, 1.4 kb; and *Hin*dIII–*Hin*dIII, 4.2 kb. Double cut *Hin*dIII/*Eco*RI and *Hin*dIII/*Bam*HI vectors were prepared from pBR322 while a *Hin*dIII/*Kpn*I vector was prepared from pSM804, a pBR322 derivative containing a unique *Kpn*I site (obtained from E. Stavnezer). Subcloning of the λ DNA fragments was by standard methods using *E. coli* DH1 (D. Hanahan, unpublished results) as the host strain.

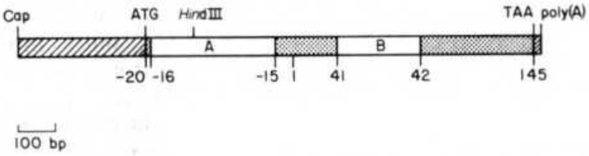

Fig. 3 Composite structure of β-HCG gene. Dotted areas represent coding regions while the cross-hatched areas represent the 5′ and 3′ untranslated regions. The positions of the two introns (A and B) are shown. The locations of the predicted cap site (Cap), the initiation codon (ATG), termination codon (TAA) and the polyadenylation site [poly (A)] are marked. Numbers at the bottom of the diagram refer to amino acids (negative numbers refer to the signal peptide). The location of the single *Hin*dIII site referred to in the text is shown. DNA fragments were labelled at their 3′ termini by DNA polymerase I (Klenow fragment; New England Biolabs) with [α-^{32}P]-labelled dNTPs (Amersham). The sequencing method of Maxam and Gilbert[5] was used.

a 3.5-kb fragment, while β-HCG 4 is on a fragment of unknown size as it is at the end of a phage λ clone. There is no evidence in the cloned isolates of the hybridizing 2.2-kb fragment seen in the filter hybridizations of genomic DNA.

Similarly, with the 5′ probe (Fig. 5) there are *Hin*dIII–*Kpn*I bands at 5.0, 4.0 and 3.7 kb. Again this correlates well with the map of the cloned genes. The 5′ ends of β-HCG genes, 2 and 4 are on 3.7-kb fragments, those of β-HCG 3, 6 and 8 are on 5.0-kb fragments, while those of β-HCG 1, 5 and 7 are on fragments of unknown size. As with the 2.2-kb fragment seen with the 3′ probe, there is no evidence in the cloned isolates of a 4.0-kb fragment hybridizing to the 5′ probe. Both of these missing fragments are seen in the filter hybridization of genomic DNA as weakly hybridizing bands and are thus good candidates for containing β-LH sequences which have not yet been detected in the phage library screening (see below).

The apparent simplicity of the filter hybridization of genomic DNA with the combined *Hin*dIII–*Kpn*I digests when compared with *Kpn*I alone is a reflection of the duplication of sequences observed in the β-HCG locus. For example, the 1.6-kb *Hin*dIII–*Kpn*I band which hybridizes strongly to the 3′ probe (Fig. 4) is known from the analysis of the bacteriophage recombinants, to contain at least four genes—β-HCG, 2, 6, 7 and 8.

The symmetrical organization of the β-HCG genes presents additional problems in correlating the cloned fragments with the bands observed in the genomic filter hybridizations. For example, after hybridization of the 3′ probe to a *Hin*dIII digest (Fig. 4), the expected 4.2-kb band is observed but at a much lower intensity than expected, as we have identified two distinct 4.2-kb fragments each of which contain two genes. In other filter hybridizations of genomic DNA (data not shown), we observed variability in the intensity of this band and attribute this to the ability of the 4.2-kb inverted fragments to form snap-back structures on renaturation during the transfer procedure. This would reduce the availability of sequences for hybridizing to the β-HCG cDNA probe and would therefore reduce the observed level of hybridization.

Because the DNA being used for the filter hybridization analysis and for the construction of the two genomic libraries came from different individuals, it is possible that the correlation of the filter hybridization data with the cloned fragments is confused by polymorphism. We have, however, found no evidence for this as the *Kpn*I and *Hin*dIII–*Kpn*I fragments that hybridize β-HCG from 10 different sources, including the DNA used to make the Charon 28 library (data not shown), are identical.

While all these factors make it difficult to determine the precise number of β-HCG-related sequences in the human genome, we believe that a reasonable estimate, based mainly on the bacteriophage λ clones we have isolated and on the *Kpn*I and *Hin*dIII digests of human DNA, is nine, of which eight have been isolated and characterized.

Are all the β-HCG genes physically linked?

Although it is not known to which chromosome the β-subunit of HCG maps, we have some evidence for the linkage of all the β-HCG hybridizing fragments. A single hybridizing band is observed in filter hybridizations of human DNA digested with either *Eco*RI or *Sal*I (our unpublished results). In the case of *Eco*RI, this fragment is between 50 and 60 kb long. Although we are unable to exclude the possibility that a band of this size contains several hybridizing fragments, which were not resolved by electrophoresis, it suggests that the β-HCG genes may all be linked. Consistent with a single hybridizing fragment is the fact that in the phage λ recombinants there are no *Sal*I sites and the only *Eco*RI sites are those two observed upstream from the 5′ end of β-HCG 6 (Fig. 1). The first of these sites may represent one end of a large *Eco*RI fragment which defines the β-HCG locus.

Despite evidence suggesting that the genes are all linked, extensive screening of two distinct genomic libraries with a β-HCG cDNA hybridization probe resulted in the isolation of 16 phage recombinants, yet failed to produce an overlapping series of sequences linking all the β-HCG genes. In fact, the isolates in the two groups *a* and *b* (Fig. 1) are remarkably similar, with clustered end points. It is possible that as there are repeats and symmetries in this locus, certain regions are unstable when cloned in *Escherichia coli*. This explanation is supported by the fact that the coding sequences tend to be found close to the ends of the phage isolates. A random distribution of DNA around the hybridizing regions would normally be expected as there are 16 independently isolated phage recombinants from two separate libraries generated by different restriction enzymes. This positioning of coding sequences at the ends of the recombinants also makes it difficult to isolate a suitable fragment for use as a probe to 'walk' along the chromosome.

One potential region of overlap between the sequences shown in Fig. 1 should be considered; it is possible that β-HCG 4 and 5 are identical and provide an overlap of ~1.5 kb between groups *a* and *b*. Some evidence in support of this overlap comes from the filter hybridizations of genomic DNA. With *Bam*HI, both probes (Figs 4, 5) identify two bands, one of ~7 kb and the other >30 kb. Linkage of the two families as proposed above would generate a 7.2-kb *Bam*HI fragment containing β-HCG 4/5 and 6. The other genes would be contained on

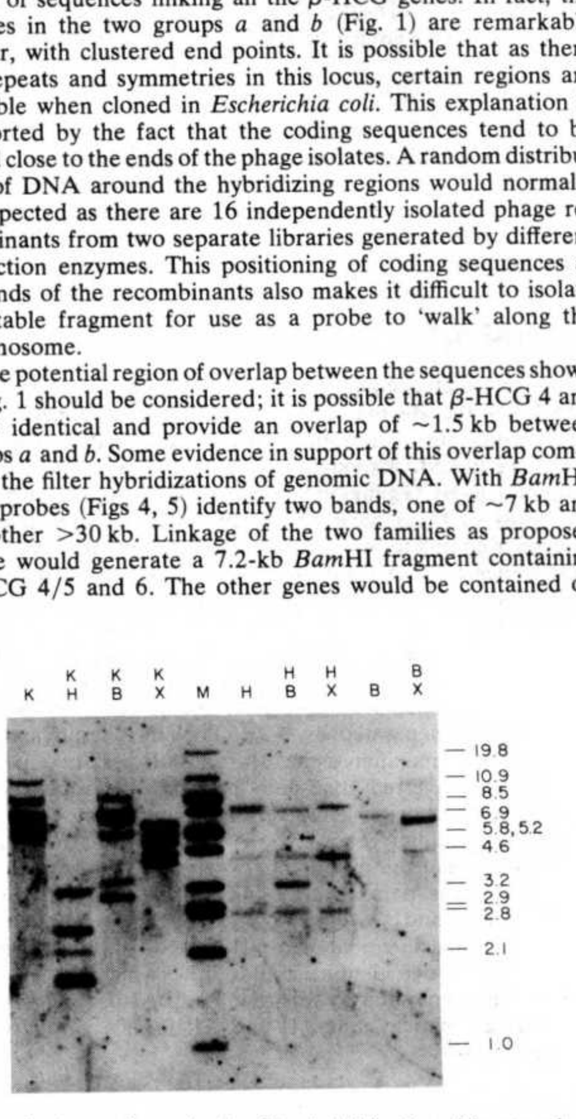

Fig. 4 Autoradiograph of a filter hybridization of human placental DNA; 10 μg of DNA were digested with various restriction enzymes, electrophoresed through a 1.0% agarose gel and transferred to nitrocellulose sheets (Schleicher and Schuell) by the method of Southern[7] as described previously[4]. The ^{32}P-labelled hybridization probe was the cloned β-HCG cDNA fragment (specific activity $1\text{–}2\times10^8$ c.p.m. μg^{-1}; ref. 3) which is specific for the region of the gene(s) to the 3′ side of the *Hin*dIII site (Fig. 3). K = *Kpn*I, H = *Hin*dIII, B = *Bam*HI, X = *Xba*I, M = markers whose sizes are given in kb at the right-hand side.

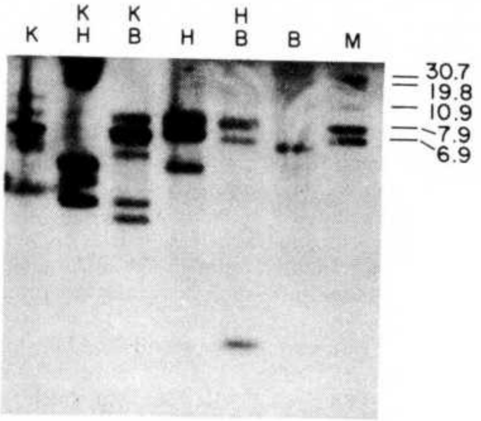

Fig. 5 Autoradiograph of a filter hybridization of human placental DNA digested with various restriction enzymes as in Fig. 4. The ^{32}P-labelled hybridization probe was a subcloned 0.8-kb *Eco*RI–*Hin*dIII fragment (Fig. 2). This probe is specific for the region of the gene(s) to the 5′ side of the *Hin*dIII site (Fig. 3). K = *Kpn*I, H = *Hin*dIII, B = *Bam*HI, M = markers whose sizes are given in kb at the right-hand side.

the large *Bam*HI fragment, which is consistent with the distribution of *Bam*HI sites in the recombinants. However, in view of the fact that the β-HCG genes and surrounding regions are repeated, this is insufficient to establish an overlap, although it is suggestive.

Discussion

We have isolated eight separate genes that hybridize to the cDNA for the β-subunit of human chorionic gonadotropin. These genes have very similar structures and, at least in some cases, are linked. Although it is difficult to estimate the total number of β-HCG genes in the human chromosome, we believe that most of them have now been isolated. Until sequence analysis is complete, we cannot exclude the possibility that the eight genes include some pseudogenes or the related gene, β-LH (see below).

Four of the β-HCG genes (1, 2 and 5, 6) have been shown to exist as inverted pairs. In both cases the genes are arranged in the order 5′–3′, space, 3′–5′ so that, assuming both genes are active, transcription would be convergent. The 3′ ends of the two genes are separated by ~2.25 kb. DNA sequencing and restriction enzyme analysis has shown that these two pairs are very similar. Inverted gene pairs have also been observed in the silk moth chorion genes, although in this case the pairs consist of dissimilar genes (type A and type B), transcription is divergent, and the distance between the 5′ ends of the two members of the pair is only ~300 nucleotides[8]. In chicken, both divergent and convergent pairs of histone genes have been identified[9]. Two dissimilar genes, H2A and H4, are convergent while there are two identical copies of H3 which are divergent, with 5′ ends separated by less than 1 kb. Also, in *Drosophila*, two different cuticle genes, II and III, form a closely linked divergent pair[10].

The other four cloned β-HCG genes are arranged in two tandem pairs; β-HCG 3 and 4 and β-HCG 7 and 8. In both cases these genes are separated by ~5.5 kb. It is also possible to consider β-HCG 2 and 3 as a divergent pair of genes with 5′ ends separated by ~8 kb. Examination of the distribution of restriction enzyme sites in Figs 1–3 reveals extensive regions of repeats: for example, the positions of the *Kpn*I, *Xba*I and *Xho*I sites preceding β-HCG 3 and β-HCG 8 are identical (Fig. 1*a* and *c*). The *Kpn*I site is almost 5 kb upstream from the proposed transcriptional start site.

The significance of the complex organization of the β-HCG genes is not obvious. The inverted and tandem pairs presumably arose from a complicated series of gene duplications and inversions encompassing an area considerably larger than the coding regions themselves. During this process the locations and sizes of the two introns remained the same. As β-HCG is the most recently evolved of the four glycoprotein hormone β-subunits, and is considered to have a common ancestor with β-LH, to which it is most closely related[1], it will be interesting to investigate the organization of the β-LH genes and to compare them with β-HCG. We consider it unlikely that the β-LH genomic organization will show the same complex patterns because the duplications and inversions of the β-HCG sequences presumably arose after the separation of β-HCG from β-LH.

In the hybridization conditions used here, it is possible that the β-HCG hybridization probes could also cross-react with β-LH as there is 82% homology in amino acid sequence between the two β-subunits[1]. Whether this cross-reactivity can be observed depends on the choice of nucleotides in the third positions of the β-LH codons. The homology of β-HCG with β-FSH or β-TSH is too low to expect any cross-hybridizaton to take place[1]. From DNA sequence and restriction enzyme analysis, we have found no evidence of β-LH gene sequences among the cloned fragments. This does not exclude the possibility, however, that β-LH is so closely related to β-HCG that one of the genes (β-HCG 1, 4, 7 and 8) for which we have no DNA sequence, could encode β-LH. It is also possible that a more faintly hybridizing band of total DNA, such as the 2.2-kb *Hin*dIII–*Kpn*I fragment (Fig. 4), which has not yet been cloned, contained β-LH sequences.

The finding that the β-subunit of HCG is coded for by several genes while the α-subunit, which is common to all four glycoprotein hormones, seems to be encoded by a single gene[4,5], is surprising. In the placenta there is an excess of free α-subunit polypeptide over β-subunit polypeptide[11], thus it might be predicted that there would be multiple α- rather than β-genes. It will be interesting to establish whether all the β-subunit genes are functional and, if so, whether there are any significant differences betwen them and between their products.

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Note added in proof: We have now established that the gene identified as β-HCG 4 is in fact β-LH (K. Talmadge and J.C.F.). This precludes the possibility that groups *a* and *b* overlap.

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LETTERS TO NATURE

Diameters and albedos of satellites of Uranus

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The five known satellites of Uranus are too small and distant to permit direct measurement of their diameters. Consequently, products of the masses of the satellites, and estimates of their bulk densities and surface albedos have been used in discussions of their probable dimensions in the absence of measurements. The presence of water ice on the surfaces of Ariel, Umbriel, Titania and Oberon has been established spectrophotometrically[1–4] and the brightnesses of the satellites have been measured photoelectrically[5]. Determination of the masses of the uranian satellites depends on observations of perturbations of the satellite orbits and results chiefly in upper limits to the products of the masses of various pairs of satellites[6,7]. The new diameter measurements reported here were made using the photometric/radiometric technique of diameter determination. This widely used technique has enabled the measurement of the diameters and albedos of approximately 250 asteroids and many planetary satellites. A recent recalibration of the technique using independent measurements of the diameters of three Solar System objects results in absolute accuracies of better than 5%. Our new albedo measurements show that Umbriel, Titania and Oberon are similar in albedo to J4 Callisto while Ariel is very similar in albedo to S7 Hyperion; the diameters of all four satellites are similar to those of the large, icy saturnian satellites S5 Rhea and S8 Iapetus.

The observations reported here were made with the 3.0-m Infrared Telescope Facility (IRTF) on Mauna Kea, Hawaii. The data were obtained on three nights in May 1982, as noted in Table 1. During this period, similar observations were made of Pluto and Neptune's satellite Triton without detections of their thermal radiation; results on these objects are given in the accompanying paper[8].

A standard broadband Q filter which transmits from 16 to 26 μm was used for all observations. The star α Boo, for which the Q magnitude was taken to be −3.32, was the primary standard[9]. Extinction on all nights averaged about 0.30 mag per airmass, which is normal for Mauna Kea; the volcanic haze covering part of the Earth's Northern Hemisphere produced no noticeable change in the extinction at 20 μm. Observations of Saturn's satellite S5 Rhea, whose radiated thermal energy distribution is similar to that of the uranian satellites, and whose diameter (1,530 ± 10 km) and photometric properties are well known from Voyager flybys[10,11], provided a consistency check on the calculated diameters of the uranian satellites. To ensure complete cancellation of the scattered thermal radiation from Uranus, the oscillating secondary mirror of the 3-m telescope was adjusted in amplitude and position angle of the oscillation so as to be perpendicular to the line joining a particular satellite and the planet. A 25-min integration on the sky diametrically opposite to the position of Ariel with respect to Uranus, where no satellite was located, resulted in a zero signal, confirming the cancellation of the flux contribution from the scattered thermal radiation of Uranus.

The calculation of diameters and albedos requires that monochromatic IR fluxes be determined from broadband observations. Because the colour temperature of the thermal radiation from airless bodies at the distance of the uranian satellites is quite low (≈80 K) and very different from that of a typical 20-μm stellar standard (≈4,000 K), close attention must be paid to the proper correction of the observed 16–26 μm flux ratios to monochromatic flux ratios at 20 μm. These corrections were made using determinations of the water vapour content of the atmosphere above the observatory derived from near IR spectra obtained on the same or adjacent nights (calibrated against a laboratory cell[12]), as well as measurements of the local atmospheric relative humidity throughout the nights made by the Kitt Peak National Observatory sky survey team. Transmission spectra of Mauna Kea model atmospheres, computed by V. Kunde for various values of precipitable water, were convolved with our filter transmission properties to derive the site-and-instrument-specific correction factors; calculated for each night because of the variability of atmospheric water vapour evidenced in the contemporaneous near-IR spectra. A detailed discussion of the monochromatic correction technique is given by Morrison[13]. The specific correction factors are listed in Table 1.

In addition to the IR observations, we have used new values of the brightnesses of the satellites in the V passband (central wavelength 0.55 μm) obtained 3 or 4 days after the IR measurements and at essentially the same solar phase angle. This angle, 0.03°, is very close to the minimum observable phase angle with the consequence that the visual magnitudes derived are some 0.4 mag smaller than published photometry[5]; in 1981 a strong, near-IR-brightness surge at opposition was discovered[3]. Since the uranian system is now almost pole-on as seen from Earth, the satellites, which are in circular orbits in Uranus' equatorial plane, are not expected to show brightness variations due to orbital motion. A detailed analysis of the opposition brightness surge will be published elsewhere[14].

For calculation of the diameters and albedos of the uranian satellites we use the standard radiometric model[13] and follow exactly the formulation used by Brown *et al.*[15]. The method is based on the determination of both reflected and absorbed

Table 1 Observations

Object	1982 dates	Integration time (min)	Q	Δ (AU)	R (AU)	Φ (deg)	H_2O (mm/airmass)	Y
Ariel	22.50 May	40	4.35 ± 0.20	17.87	18.88	0	≈1.0	0.88
Umbriel	20.33 May	40	4.67 ± 0.15	17.87	18.88	0	2–3	1.10
Titania	20.50 May	30	3.96 ± 0.10	17.87	18.88	0	2–3	1.10
Oberon	19.40 May	50	3.93 ± 0.14	17.87	18.88	0	3–4	1.20
Rhea	17.25 May	25	1.27 ± 0.04	8.88	9.68	4	2–3	1.03
Rhea	22.40 May	25	1.19 ± 0.04	8.93	9.68	4	≈1.0	0.93

Q is the broadband 20-μm magnitude. Δ is the object–Earth distance. Φ is the solar phase angle. H_2O is the precipitable water vapour. Y is the monochromatic correction factor (to be multiplied by the observed flux to yield the monochromatic flux). All errors quoted are ±1 s.d. of the mean.

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Table 2 Calculated diameters and albedos

Object	m_{20}	$V(0°)$	$V(1.0)$	q	Diameter (km)	p_v
Ariel	-1.78 ± 0.22	1.03 ± 0.05	1.3 ± 0.2	0.8 ± 0.1	$1,330\pm130$	0.30 ± 0.06
Umbriel	-1.70 ± 0.18	1.89 ± 0.05	2.2 ± 0.2	0.7 ± 0.1	$1,110\pm100$	0.19 ± 0.04
Titania	-2.41 ± 0.14	0.91 ± 0.05	1.2 ± 0.2	0.7 ± 0.1	$1,600\pm120$	0.23 ± 0.04
Oberon	-2.53 ± 0.17	1.10 ± 0.05	1.4 ± 0.2	0.7 ± 0.1	$1,630\pm140$	0.18 ± 0.04
Rhea	-3.54 ± 0.06	—	0.24	1.02	$1,530\pm45$	0.60 ± 0.04
Rhea	-3.53 ± 0.06	—	0.24	1.02	$1,530\pm45$	0.60 ± 0.04

m_{20} is the 20-μm monochromatic magnitude corrected to an Earth–object distance of 1 AU and 0° solar phase angle (assuming a brightening of 0.01 mag deg^{-1}). $V(0°)$ is the visual magnitude at 0° solar phase angle corrected to 1 AU from the Earth and Sun (see Text). $V(1, 0)$ is the $V(0°)$ with the opposition surge removed (see text), q is the assumed phase integral (except the value for Rhea taken from ref. 11). p_v is the visual geometric albedo. All errors are ±1 s.d. of the mean, except those quoted for the assumed phase integrals and the $V(1, 0)$, which represent our estimates of a reasonable range of values.

solar radiation. For an airless, slowly rotating body, we assume that the surface is in approximate thermal equilibrium with the absorbed component of the incident energy. The absorbed power is reradiated and can be determined from a measurement of thermal IR emission, such as the 20-μm observations reported here, while the reflected power can be determined by conventional photometry.

With observations of reflected and reradiated solar energy, it is possible to obtain a solution for albedo and diameter that is only weakly dependent on assumed parameters, such as the detailed photometric or thermophysical properties of the observed objects. In the case of the uranian satellites, possible departures from thermal equilibrium due to rotation will be unimportant, as the satellites are nearly pole-on with respect to the Earth and Sun. As previously mentioned, a consistency check is provided by the observations of Rhea, an object of known diameter and photometric properties, with a surface temperature distribution similar to that of the uranian satellites. (For more complete descriptions of the method see refs 13, 16, 17, and 15 for details of the mathematical formulation and error analysis.)

The phase integrals of the uranian satellites cannot be measured from Earth and a plausible value must be assumed for each satellite to derive its diameter and albedo. Jupiter's satellite Callisto ($p_v = 0.19$) has a measured phase integral of 0.7 (ref. 18), which we consider appropriate for use in the reduction of the Umbriel, Oberon, and Titania data in view of their roughly equivalent albedos. As the albedo of Ariel is higher, a phase integral of 0.8 (a value measured for Jupiter's Ganymede[18]) was adopted. However, for relatively dark objects, the method of reduction to diameters and albedos is only weakly dependent on the choice of a phase integral. For the case of Ariel, a change of ±0.1 in the assumed phase integral of 0.8, results in about a ±3% change in the derived diameter; for Umbriel, Titania, and Oberon, a change of ±0.1 in their assumed phase integral of 0.7 results in a ±2% change in the derived diameters. The estimated uncertainties in the phase integral have been incorporated into the quoted uncertainties for the diameters and albedos of the four satellites.

As previously mentioned, the visual magnitudes $V(0°)$ listed in Table 2 were obtained at a solar phase angle of 0.03° and the therefore contain a substantial opposition surge. The calibration of the radiometric diameter scale by Brown *et al.*[15] uses visual magnitudes of the calibration objects which do not include the average opposition surge of ≈0.3 mag (ref. 19). To ensure that the diameters of the uranian satellites are calculated with photometric parameters more closely analogous to those of the Brown *et al.* calibration objects, visual magnitudes 0.3 mag fainter than the $V(0°)$ in Table 2 were used in the reduction to diameters. Since the resulting albedos of the uranian satellites are relatively low, the derived diameters will depend only weakly on the visual magnitude[13]. In contrast, the derived albedos are very sensitive to the visual magnitude and the large opposition surges of the uranian satellites imply that their albedos are a strong function of the viewing geometry. For the purpose of calculating the albedos seen at an arbitrary solar phase angle, the satellite diameters should henceforth be considered independent of visual magnitude. The visual magnitudes used in the reduction to diameters are listed in Table 2 in the column $V(1, 0)$. The error bars estimated for the diameters include the tabulated uncertainty in $V(1, 0)$.

The diameters and albedos shown in Table 2 are the first measurements of these important quantities for any of the uranian satellites. Previous estimates of the diameters of the satellites of Uranus are based on, and very sensitive to, assumptions regarding the satellites' albedos, and, except those of a recent spectrophotometric study[4], tended to underestimate the satellites' sizes (It is interesting that the newly measured albedos agree with those inferred from a proposed relation between albedo and *VJHK* colour indices among outer Solar System bodies[20]). Ariel, Umbriel, Titania and Oberon all have diameters roughly similar to the large, icy, Saturn satellites, particularly S5 Rhea, but significantly lower albedos (Rhea's albedo is about 0.6) than all known saturnian satellites except Hyperion, Phoebe, and the dark side of Iapetus. Hyperion has an albedo of 0.3, roughly equal to the albedo of Ariel, and reflectance spectra of Ariel and Hyperion in the 1.5–2.5 μm region are strikingly similar[3,21]. The relatively low albedo of Hyperion and its reddish appearance in Voyager images suggests the presence of a dark contaminant on/in its surface; Hyperion's spectral similarity to Ariel may indicate that the surface contaminants are similar in both bodies. This argument can be extended by analogy to Umbriel, Titania, and Oberon[3].

A comparison with bodies in the jovian system shows that the albedos of the uranian satellites are intermediate to the albedos of Ganymede and Callisto, whose surfaces are known to be largely composed of water ice contaminated with darker particles[22]. The relatively low albedos of the uranian satellites suggest that a dark contaminant is present on/in their surfaces, though there may be significant differences in the degree of contamination, the chemistry of the contaminant, the details of intermixing and the microstate of the surfaces when compared with the jovian and saturnian satellites. The question of the nature of the contaminant(s) in the surfaces of the uranian satellites is being addressed by one of us (R.H.B.), and will be published elsewhere.

Table 3 Satellite masses

Object	Mass (10^{-5} Uranus) $\bar{\rho} = 1.0$ g cm^{-3}	$\bar{\rho} = 1.3$	$\bar{\rho} = 2.3$
Ariel	1.42	1.85	3.27
Umbriel	0.83	1.08	1.91
Titania	2.48	3.22	5.70
Oberon	2.62	3.41	6.03

The satellite mass is shown for different values of an assumed mean density. The mass units are 10^{-5} Uranus mass where the mass of Uranus is 8.66×10^{25} kg. A density of 1 g cm^{-3} is approximately that of pure water, a density of 1.3 g cm^{-3} corresponds to ~60% water ice and 40% silicates by weight and 2.3 g cm^{-3} is typical of carbonaceous chondritic material.

Table 3 lists the masses of the uranian satellites assuming several values for the mean density of each satellite and using the radii from this study. From observations of orbital perturbations, Greenberg[6,7] has derived an upper limit to the product of the masses of Ariel and Umbriel of $\leq 10^{-9} M^2$ (M = the mass of Uranus). If we assume that the mean densities ($\bar{\rho}$) of Ariel and Umbriel are approximately equal, and use the radii from this study, we find that the Greenberg upper limit is satisfied for $\bar{\rho} \leq 2.91$ g cm^{-3}. Veillet and Rattier[23] have reported that new observations and analyses of Miranda's motion yield a value for the product of the masses of Ariel and Umbriel of $1.10 \pm 0.25 \times 10^{-10} M^2$ which, with our radii, is consistent only with mean densities of ≤ 1 g cm^{-3} for both satellites. The same study yields $1.5 \times 10^{-4} M$ for the mass of Titania. This value with our radius yields a mean density of 6.0 g cm^{-3}, which we believe is unreasonably high for a satellite of mainly ice composition. Densities of ≈ 1.3 g cm^{-3}, typical of Saturn's icy satellites[24], and the densities derived from Greenberg's Ariel–Umbriel mass constraints are consistent with the observed icy character of the surfaces of the uranian satellites. Determination of the mean densities of the satellites is crucial to the understanding of the origin and subsequent evolution of the uranian system. With reliable measurements of the uranian satellite radii now in hand, observations from which the satellite masses can be derived become paramount.

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Diameters of Triton and Pluto

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Triton, the major satellite of Neptune, and Pluto, the outermost planet, are too small and distant to permit direct measurement of their size, and indirect arguments must be used to estimate diameter and albedo. These two objects invite a comparative study because they have essentially the same brightness (absolute visual magnitude $V(1,0) \simeq -1$) and current distance from the Sun (30 AU). In addition, the presence of methane frost on the surface, and of a possible methane atmosphere, have been detected for both objects from IR spectroscopy[1–4]. On the basis of these similarities, Triton and Pluto were selected as targets for possible detection of thermal IR emission. We report here new upper limits to the thermal IR emission from these objects that permit us to set significant upper limits on their diameters and to demonstrate that both are high albedo objects. These results clearly exclude the possibility that Triton is the largest planetary satellite, and they are consistent with the small size of Pluto deduced from other data.

Our observations were made using a Ge bolometer at the 3.0-m Infrared Telescope Facility (IRTF) on Mauna Kea. This instrument, located at an elevation of 4.2 km, is among the most sensitive in the world for observations in the 20–40 μm spectral region where thermal radiation from these objects should be most easily detected. Observations were made on three nights (19, 20, and 22 May 1982) under photometrically clear skies. Atmospheric water vapour, as monitored from 1 to 3 μm spectrometry, declined over this observing period from about 3 mm precipitable H_2O to <1 mm. We were concerned about the effect on the observations of the worldwide high-altitude volcanic haze, which was dramatically apparent near sunrise and sunset and produced anomalously high visible extinction, but the performance of the IRTF at 20 μm appeared to be unimpaired by this dust cloud.

All observations were made through a standard broad-band Q filter, covering wavelengths from ~16 to 26 μm. Stellar standards, and their adopted Q magnitudes, were α Lyr ($Q = -0.10$) and α Boo ($Q = -3.32$) (refs 5, 6). Measured stellar extinction coefficients were in the range 0.2–0.4 mag per air mass. As an additional standard with a spectral energy distribution more like that of Triton and Pluto, we selected Saturn's satellite S5 Rhea. Other programme objects observed on these nights were the uranian satellites Oberon, Titania, Umbriel, and Ariel; the results for these satellites together with additional details of the observing procedures are reported by Brown *et al.*[7] in an accompanying paper.

Pluto was observed in excellent conditions on 20 May for a total of 4,200 s. Triton, which is at a more southerly declination and is therefore viewed through larger air masses, was observed for a total of 13,800 s, approximately equally distributed over three nights. Neither object was detected.

The observations are summarized in Table 1. The signal and noise levels are given in units of the signal through the Q filter from a star of magnitude $Q = 0$, as determined from the observations of α Boo and α Lyr. Each 'observation' consists of a 300-s integration period, made up of 15 pairs of 10-s integrations, with the programme object centred alternately in each beam. Each 300-s data block generates a value for the signal and for a standard deviation in the mean computed from the 15 individual integrations. These observations are then corrected for extinction and averaged to yield a single value for each night. The uncertainty is calculated both from the standard deviation of the 14–16 individual observations and from the average of the individual observations divided by $\sqrt{N}$, where N is the number of observations (14 or 16). These two estimates of uncertainty agreed well in all cases, demonstrating freedom from nonstatistical sources of error.

The final Q-band values for Triton and Pluto are given in Table 1. For Pluto we obtain $(3 \pm 20) \times 10^{-4}$ where the indicated uncertainty is the mean of the two values calculated. For Triton the final value is $(15 \pm 18) \times 10^{-4}$. In these units, a flux of

Table 1 IR observations 17–22 May 1982

Object	$V(1, 0)$	UT date	N*	Q-band signal†	m_{20}‡
Rhea	+0.24	17 May	5	0.31 ± 0.02	1.2
		22 May	5	0.34 ± 0.03	1.2
Pluto	−0.7	20 May	14	$(3 \pm 20) \times 10^{-4}$	7.1
Triton	−1.0	19 May	14	$(83 \pm 40) \times 10^{-4}$	7.2
		20 May	16	$(8 \pm 25) \times 10^{-4}$	
		22 May	16	$(-13 \pm 25) \times 10^{-4}$	

* Number of observations of 300 s each.

† Signal relative to that of a star with $Q = 0$.

‡ Monochromatic 20-μm magnitude (corresponding to 1σ values for Pluto and Triton).

20×10^{-4} is equivalent to magnitude $Q = +6.8$. For both objects, the 3σ upper limit is $Q = +5.3$.

For a planetary object in approximate thermal equilibrium with the incident sunlight, the diameter and albedo can be calculated from two quantities: the absolute visual magnitude $V(1, 0)$ and the monochromatic 20-μm magnitude m_{20} (refs 8, 9). We present results of these calculations for both the 1σ and 3σ values of Q given above.

The values of $V(1, 0)$ for Pluto and Triton are obtained from previous photometry and will be used here without correction for possible dependence on solar or orbital phase angles. For Pluto, we adopt the value of $B_0 = 15.98$ obtained in 1980 by Tedesco and Tholen[10]. For a colour $B - V = 0.79$ and a mean distance 39.4 AU, this value of B_0 implies $V(1, 0) = -0.7$. This absolute magnitude is fainter than that generally used in the past, reflecting the gradual decline in brightness of Pluto observed in recent years[1]. For Triton, we use the value $V(1, 0) = -1.0$ obtained in 1972 and 1977 by Degewij *et al.*[11].

The Q magnitudes obtained in our observations are converted to monochromatic magnitudes (m_{20}) using the methods described by Morrison and others[8,12]. We have calculated the sensitivity of our photometer as a function of wavelength over the Q filter band for a variety of atmospheric transmission functions, depending on the humidity. The corrections were made separately for each night, using the depth of the 1.4-μm telluric water band and the value of the Q-magnitude extinction coefficient as indicators of the absorption by telluric H_2O in the Q band. The calculated values of m_{20} are given in the final column of Table 1. The 1σ and 3σ values of m_{20} adopted for our calculations are 7.1 and 5.6, corresponding to 20-μm fluxes of 0.014 and 0.054 Jy, respectively.

The diameters and albedos were calculated from these magnitudes using the recent small revisions in the radiometric diameter scale by Brown *et al.*[12]. We adopted for these calculations a phase integral $q = 1.0$, appropriate for objects with moderately high albedo. As a check, we used the same methodology to calculate the diameter of Rhea from observations made at the same time. For Rhea we obtain $R = 765 \pm 25$ km, in good agreement with the Voyager value of $R = 765 \pm 5$ km, for this satellite[13].

For Triton, the 1σ limit yields $R = 1{,}800$ km and albedo 0.43, while the 3σ values are $R = 2{,}100$ km and 0.25. For Pluto, the 1σ limit yields $R = 1{,}500$ km and albedo 0.37, the 3σ values are $R = 1{,}950$ km and 0.22.

The diameter of Triton has long been a subject of speculation but of few firm data. Because its absolute brightness [$V(1, 0)$] is comparable to that of Titan and Callisto, it has frequently been noted that Triton could, in fact, be the largest of the satellites, if its albedo were low (<0.1). In 1954 Kuiper attempted to measure its size directly with the 5-m Hale telescope, estimating $R = 1{,}900$ km (ref. 14), but the uncertainty in this value was both large and poorly defined[15]. In reviews published in 1974 and 1977[16,17] only upper and lower limits to the radius of 6,500 and 1,100 km, respectively, were listed based on plausible albedos.

In 1978 Cruikshank *et al.*[15] presented spectroscopic evidence that the surface of Triton was 'rocky', leading them to estimate that the albedo was confined to the range $0.02 \leq p_V \leq 0.5$, corresponding to radii between 7,400 and 1,500 km. These authors also used the radiometric technique to set a 3σ limit of $m_{20} > 4.3$, constraining the radius to be smaller than ~2,600 km, which is very near to the radius of the largest satellites: Ganymede, Callisto, and Titan. Thus their final estimate for the radius of Triton was $2{,}600 \geq R \geq 1{,}500$ km.

The 3σ upper limit for the radius of Triton set in this study is 2,100 km, 80% of the limiting value obtained by Cruikshank *et al.*[15]. To estimate a lower limit, we reason by analogy with other outer Solar System satellites. With but one exception, the icy satellites of Jupiter and Saturn have albedos ≤ 0.7. (Enceladus, with $p_V \simeq 1.0$, is an exceptional object, apparantly subject to a long history of surface activity)[18]. An additional constraint is suggested by the downturn in Triton's spectral reflectivity in the UV, which resembles that of Europa ($p_V \simeq 0.6$). We adopt here a limiting albedo $p_V < 0.7$, corresponding to $R > 1{,}300$ km.

The range of sizes for Triton quoted above ($2{,}100 > R > 1{,}300$ km) represents limits, not standard errors in the usual statistical sense. Our conclusions about the size and albedo of Triton, presented in the more conventional form of a probable value and standard deviation, would be $R = 1{,}600 \pm 200$ km, $p_V = 0.4 \pm 0.1$. This is the same size range as Io and Europa, and it is clearly smaller then the giant satellites Ganymede, Callisto, and Titan. The albedo is that of an icy surface with no more than moderate contamination, roughly similar to Ganymede in the jovian system or to the major icy satellites of Saturn, but distinctly brighter than the uranian satellites.

The size of Pluto is unknown, although its mass is fairly well constrained by the known parameters of the orbit of Charon, Pluto's satellite. The first indication that Pluto might be a lunar-sized object ($R \sim 2{,}000$ km) was derived from the detection of methane ice on its surface, suggesting that it probably had a moderately high albedo[1]. From its mass of 0.0022 Earth masses [19] and the physical constraint that its density be unity or greater, a radius of $R < 1{,}460$ km is implied. However, recent speckle interferometric results[20,21] give radii in the range 1,500–2,000 km. A radius in the middle or upper end of this range would lead to a density that appears implausibly low, but all the data are consistent with a radius ~1,500 km.

The 3σ upper limit to the size of Pluto derived from our observations is $R < 1{,}950$ km, while a more probable upper limit is ~1,700 km, consistent with the other estimates quoted above. The albedo of Pluto for $R = 1{,}500$ km and $V(1, 0) = -0.7$ is 0.4, similar to that judged most likely for Triton and rather typical of icy satellites in the jovian and saturnian systems, but not of the uranian satellites.

The radiometric upper limits derived in this paper for the diameters of Triton and Pluto are consistent with icy objects of sub-lunar size, and with the hypothesis that the two objects are physically similar to each other. If, as we suspect, they both have albedos ~0.4, then about a factor of two improvement in IR systems will be required to measure their thermal emission.

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Flow and instability of a viscous current down a slope

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If viscous fluid is released on a horizontal surface it rapidly takes up a circular plan form as it spreads. This form is observed[1,2] to be stable to any small disturbances which are initiated on the front due, for example, to irregularities in the horizontal surface or to chance perturbations. Alternatively, if some fluid is released onto a sloping surface—for example, some liquid detergent on a slanted plate—a quite different plan form occurs. One, two or more extended regions of fluid develop downslope, as shown in Fig. 1*a*, *b*. A situation intermediate between these two is now discussed. Consider a broad band of viscous fluid, uniform in depth across a slope, released so as to flow down a constant slope. By following the motion, which is initially independent of the cross-slope coordinate, the speed of advance and the depth of the flow before it breaks up into a series of waves of ever increasing amplitude can be determined. I present an expression for the wavelength of the front, which is determined by surface tension and is independent of the coefficient of viscosity.

With the coordinate system depicted in Fig. 2 and use of the approximations of lubrication theory[3], the y-independent, down-slope momentum equation can be written as

$$0 = g\sin\alpha + \nu u_{zz} \tag{1}$$

In deriving equation (1) we have neglected both surface tension, the effects of which will be analysed below, and contact line effects[4], which are negligible[1] under the assumption that the Bond number $B = \rho g l^2/T \gg 1$, where ρ is the fluid density, l a representative length scale of the current and T the surface tension. Use of the equation of continuity then leads to

$$h_t + (g\sin\alpha/\nu)h^2 h_x = 0 \tag{2}$$

as the nonlinear partial differential equation for the unknown free surface $h(x, t)$. To equation (2) must be added the global continuity equation

$$\int_0^{x_N(t)} h(x,t)\mathrm{d}x = A \tag{3}$$

where $x_N(t)$ is the value of x at the front of the current and A is the initial cross-sectional area. The particular solution of equations (2) and (3) is sought in the range $0 \leqslant x \leqslant x_N(t)$.

Equation (2) shows that h is constant along characteristics given by

$$\frac{\mathrm{d}x}{\mathrm{d}t} = (g\sin\alpha/\nu)h^2 \tag{4}$$

Thus if initially $h = f(x)$, say, the equation of the characteristics is given by

$$x = x_0 + (g\sin\alpha/\nu)f^2(x_0)t \tag{5}$$

where x_0 is the initial value of the characteristic. The solution of equation (2) is thus

$$h = [\nu(x-x_0)/g\sin\alpha]^{1/2}t^{-1/2} \tag{6a}$$

$$\to (\nu/g\sin\alpha)^{1/2}x^{1/2}t^{-1/2} \qquad (x \gg x_0) \tag{6b}$$

independent of the initial conditions. When combined with equaton (3), in order to evaluate the length of the current, equation (6*b*) proves that some time after the initiation of the current, no matter what the initial shape, the solution takes the form

$$\begin{gathered} h = (\nu/g\sin\alpha)^{1/2}x^{1/2}t^{-1/2} \\ 0 \leqslant x \leqslant x_N = (9A^2 g\sin\alpha/4\nu)^{1/3}t^{1/3} \end{gathered} \tag{7}$$

Expressed alternatively, equation (7) represents the unique similarity solution of equations (2) and (3). The profile of the predicted current ends abruptly at $x = x_N$ with $h = h_N(t) = 1.5A/x_N$ there. The profile can be smoothed off at x_N by including the effects of surface tension. This will be done below after a discussion of an experimental investigation of the validity of the truncated profile expressed by equation (7).

Some 30 experiments were conducted using a pentagonal sheet of Perspex. The longest side was 101.7 cm and from it two sides of length 82 cm emanated at right angles. The remaining two sides were 60 cm. The sheet was surrounded by a Perspex wall 5 cm high to make a tray which was firmly attached to a rigid, flat board. Before each experiment the board was tilted by raising the 101.7-cm long side by the required amount. A removable Perspex gate was fitted 5.0 cm from the back edge of the tray and fluid poured into the space behind the gate. Three different fluids, whose physical properties are listed in Table 1, were used. After the fluid behind the gate had been left a sufficiently long time that it had become quite stationary, the gate was raised and the fluid proceeded to pour down the slope.

At first the motion was virtually independent of the cross-slope coordinate except near the walls where viscous drag retarded the flow. This form of motion is clearly evident in Fig. 1*c*. Observations of the length of the current as a function of time were taken and some typical results plotted in Fig. 3. It can be seen that the agreement between the experimental observations and the theoretical prediction (7) is good. The agreement justifies the neglect of both surface tension and contact line effects in predicting the temporal development of the two-dimensional current.

After the two-dimensional motion had continued for some time, the flow front seemed spontaneously to develop a series of small amplitude waves of fairly constant wavelength across the slope, as seen in Fig. 1*d*. The amplitude of the waves increased in time as the maxima (points furthest down the slope) travelled faster than the minima, as seen in Fig. 1*e*. The wavelength remained unaltered. The long-time shape of the flow front was different for the two fluids used. Both silicone oils developed the form shown in Fig. 1*f*. This consisted of a periodic, triangular front with tightly rounded maxima. The maxima were connected by very straight portions at an angle to the slope to extremely pointed minima. The glycerine developed the form shown in Fig. 1*g*. This was also periodic, though with much less tightly rounded maxima. These were again connected by extremely straight portions, but in this case they were almost directly down slope and connected to very broad minima.

Table 1 The fluids used, their viscosity and surface tension

Fluid	Viscosity at 17 °C ($\mathrm{cm^2 s^{-1}}$)	Surface tension at 17 °C ($\mathrm{dyn\ cm^{-1}}$)
Silicone oil MS200/100	1.15	20.6
Silicone oil MS200/1000	12.8	22.0
Glycerine	9.8	60.2

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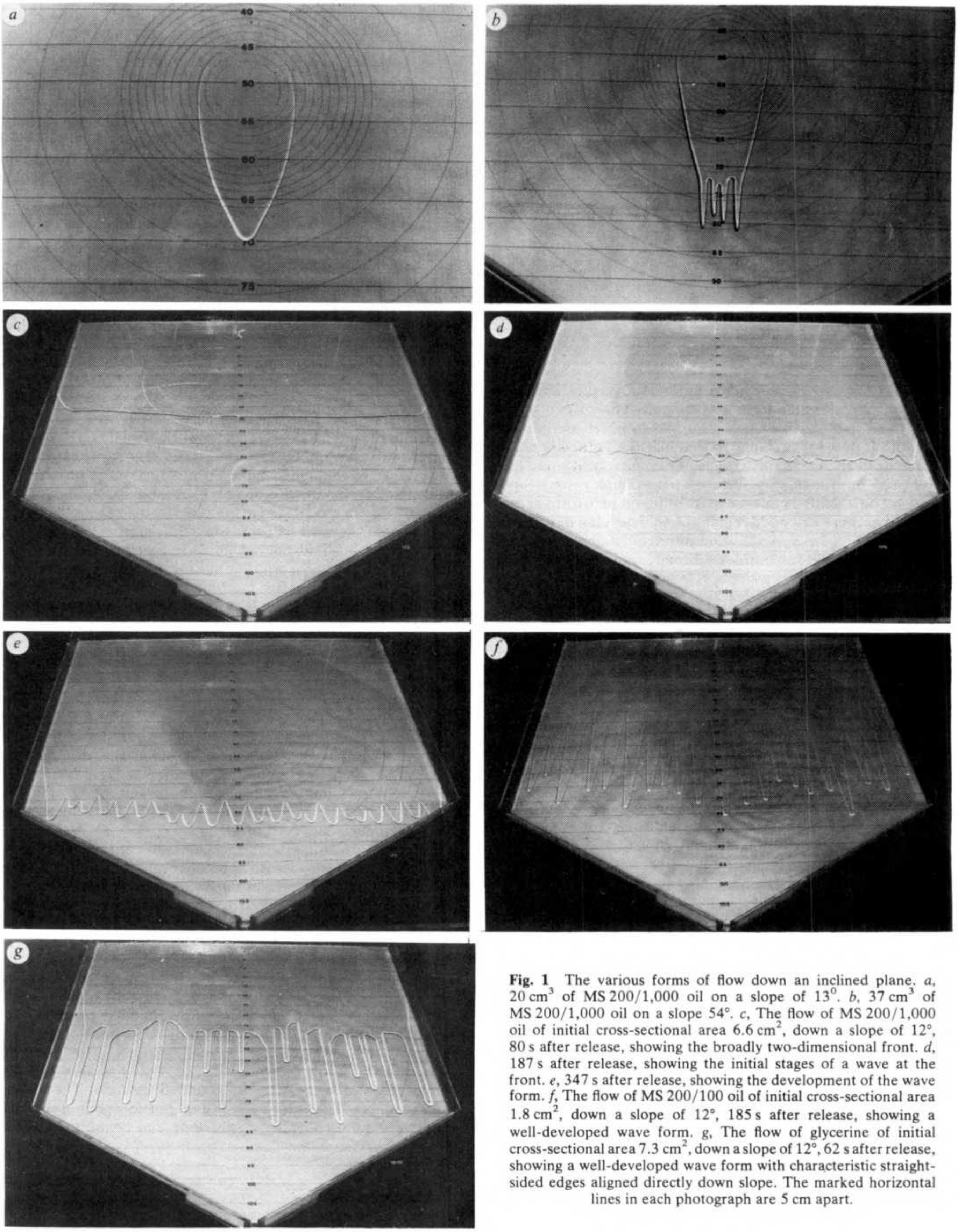

Fig. 1 The various forms of flow down an inclined plane. *a*, 20 cm^3 of MS 200/1,000 oil on a slope of 13°. *b*, 37 cm^3 of MS 200/1,000 oil on a slope 54°. *c*, The flow of MS 200/1,000 oil of initial cross-sectional area 6.6 cm^2, down a slope of 12°, 80 s after release, showing the broadly two-dimensional front. *d*, 187 s after release, showing the initial stages of a wave at the front. *e*, 347 s after release, showing the development of the wave form. *f*, The flow of MS 200/100 oil of initial cross-sectional area 1.8 cm^2, down a slope of 12°, 185 s after release, showing a well-developed wave form. g, The flow of glycerine of initial cross-sectional area 7.3 cm^2, down a slope of 12°, 62 s after release, showing a well-developed wave form with characteristic straight-sided edges aligned directly down slope. The marked horizontal lines in each photograph are 5 cm apart.

The instability cannot be explained by viscous effects alone, since any cross-slope variation in depth of the current tends to be diminished, that is stabilized, by the cross-slope pressure gradient the variation induces. Surface tension must be taken into account.

Experiments with silicone oils whose coefficients of viscosity differ by an order of magnitude, although their surface tensions are almost equal, indicated that the wavelength of the instability is independent of the coefficient of viscosity. Experiments with the two fluids of comparable viscosity yet different surface

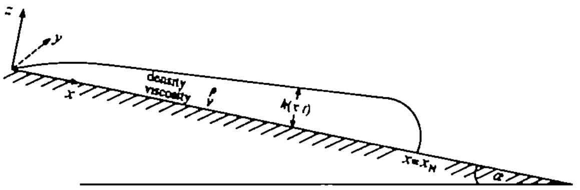

Fig. 2 A sketch of the flow and coordinate system.

tensions indicated that the wavelength is a function of the surface tension. Finally, observations taken during experiments using the same fluid but with amounts differing by up to a factor of 10 indicated that the wavelength is weakly dependent on the initial cross-sectional area A.

The form of the quasi-steady, two-dimensional tip, which is determined by including surface tension and by matching the tip onto the main flow given by equation (7), can be easily determined. The addition to equation (2) of the terms due to surface tension T leads to

$$h_t + (g\sin\alpha/\nu)h^2h_x - 1/3(T/\rho\nu)h^3h_{xxxx} = 0 \tag{8}$$

In the tip the dominant balance is between the second and third terms of equation (8), the solution to which can be written as

$$h = h_N(t)H(\xi) \qquad \xi = (\rho g\sin\alpha/Th_N)^{1/3}(x_N - x) \tag{9}$$

where $H(\xi)$ satisfies

$$H^3H''' + H^3 = 1 \tag{10}$$

$$H \to \left(\frac{16}{15}\right)^{1/4}\xi^{3/4} \qquad (\xi\to 0) \tag{11}$$

$$\to 1 \qquad (\xi\to\infty) \tag{12}$$

The length scale of the tip is thus given by $(Th_N/\rho g\sin\alpha)^{1/3}$.

The experiments indicated that the instability occurs after the two-dimensional flow has propagated to a critical length X_N^C proportional to $A^{1/2}$. Evaluating the length-scale of the tip at this point and conjecturing that the wavelength, λ, is given, in scale, by this length, suggests that $\lambda \sim (A^{1/2}T/\rho g\sin\alpha)^{1/3}$. The results of the various observations, normalized in this way, are presented in Fig. 4. The data are seen to collapse together systematically with this normalization and are well represented by

$$\lambda = 7.5(A^{1/2}T/\rho g\sin\alpha)^{1/3} \tag{13}$$

The wavelength remained constant as the amplitude of the instability increased. For the silicone oils, the distance from the origin of the furthest points down the slope increased with time like $t^{0.35}$ while the distance of the minima increased like $t^{0.28}$. For glycerine, the distance of the front of the instability, once well established, increased like $t^{0.6}$ while the minima were virtually stationary.

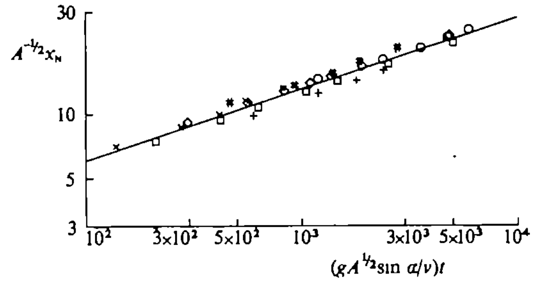

Fig. 3 The length of the two-dimensional flow, normalized with respect to $A^{1/2}$, as a functon of a suitably non-dimensional time. The straight line is the theoretical prediction obtained from equation (7). The experimental points are for values of ν, $\sin\alpha$, A in cgs units of: □, 12.9, 0.093, 8.9; ◇, 12.9, 0.20, 3.1; ○12.9, 0.65, 5.9; +, 9.8, 0.20, 9.0; ×, 9.8, 0.15, 3.6; #, 1.2, 0.11, 1.0.

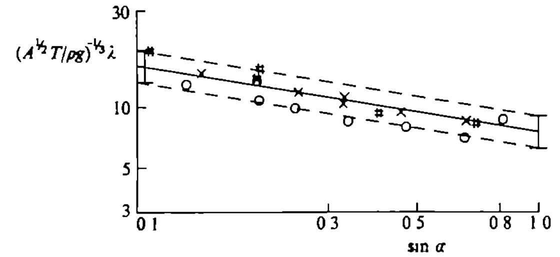

Fig. 4 The wavelength of the instability at the front, normalized with respect to $(A^{1/2}T/\rho g)^{1/3}$, as a function of the slope angle, α. Experiments with fluids of viscosities 12.9, 9.8 and 1.2 cm^{2}s^{-1} are plotted as ○, × and # respectively. The solid line is given by equation (14) and is the best-fit to the data. The two error bars at the end of the line represent ±15%, a typical standard deviation of the wavelength across the slope. Where two or more data points fell on top of each other all but one were omitted for the sake of clarity.

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Forces between two adsorbed polyethylene oxide layers immersed in a good aqueous solvent

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Recent measurements of the forces acting between two polystyrene layers adsorbed onto mica surfaces, immersed in a poor solvent for the polymer, show strong initial attraction as the surfaces approach, followed by ultimate repulsion[1]. This has been attributed to attractive osmotic interactions between the adsorbed layers, and possibly to the effect of bridging[2,3]. We have extended these measurements to the case of polyethylene oxide (PEO) layers adsorbed on mica, immersed in an aqueous 0.1 M KNO_3 medium at pH 6 (a good solvent for PEO). Using monodispersed polymer of two molecular weights, we find that an equilibrium force–distance profile is indicated. As the surfaces bearing the adsorbed PEO approach, repulsive forces commence at a surface separation $D \simeq 6\pm1R_g$ (unperturbed radius of gyration of the respective polymers) and increase monotonically on approach; on subsequent separation the forces decrease monotonically to zero. We find no evidence for attraction or adhesion between the adsorbed layers in this system.

The experimental technique and procedure closely resemble those previously described[1,4]. The method permits the measurement of the force $F(D)$ acting between the mica surfaces a distance D apart, as well as the mean refractive index $n(D)$ of the medium separating them.

The forces were first determined between the bare mica surfaces in pure electrolyte. Polyethylene oxide solution was

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then added to the required concentration (either 10^{-5} or 1.5×10^{-4} g ml^{-1}), and the surfaces left to incubate for 16±2 h. Forces were then measured both in compression and decompression at different intervals (1 min to 1 h) and at various approach and separation rates (15–60 min per cycle), following which the PEO solution was replaced by pure solvent, and measurements repeated. The overall duration of experiments was generally between 48 and 72 h.

The PEO samples used were obtained from Toyo Soda (Japan). They were anionically prepared and their molecular characteristics (manufacturers data, determined by GPC) are shown below.

	M_w	M_w/M_n	R_g
PEO1	4.00×10^4	1.03	6.5 nm
PEO2	1.60×10^5	1.04	13 nm

The results are shown in Fig. 1. In the pure electrolyte (Fig. 1*a*) the forces between the mica surfaces are typical of electrostatic double-layer (DLVO) interactions[4], with a secondary minimum as indicated in the inset and the characteristic strong short range repulsion (Fig. 1*a*). The surface potential of the mica deduced from the Debye length is ~100 mV. The force–distance profiles as well as the surface potential correspond closely to those observed previously for mica surfaces in various aqueous electrolytes[4].

Following adsorption of the polymer, the force profiles change as shown in Fig. 1*a*: no forces are observed as the surfaces approach from $D\simeq500$ nm down to $D\simeq6\pm1\,R_g$ (corresponding to 35–40 nm for PEO1, 65–75 nm for PEO2), and thereafter monotonically increasing repulsion sets in. The surfaces were generally brought in to about 6±1 nm separation. The shape of $F(D)$ on decompression and recompression of the surfaces depends to some extent on the rate at which these are carried out: if decompression is rapid (up to about 5 min for separation to $F(D)=0$) the dashed curve (Fig. 1*a*) is followed. However, if separation is sufficiently slow (30–60 min for separation to $F(D)=0$), the decompression curve merges (within error) into the original compression profile (solid curve). A similar effect is observed on recompression: if the surfaces are compressed, then rapidly separated, (<5 min) and then brought together again within 5 min, then the resulting $F(D)$ profile on subsequent compression follows the broken curve. If a longer time (30–60 min) elapses between separation and approach, the compression profile reverts to the original solid curve. Thus the variation of $F(D)$ with compression–decompression rates in these experiments is within the shaded regions indicated. We find no evidence for the build-up of thick adsorbed layers with time beyond the values corresponding to the solid curves in Fig. 1. The results described were obtained (within experimental error) for the two incubation concentrations used, both in solution and following the replacement of polymer solution by pure electrolyte. They suggest that there is a quasi-equilibrium force–distance profile for this system, represented by the solid curves, while the broken curves indicate the effect on $F(D)$ as a result of incomplete relaxation of the adsorbed polymeric layers at short times ($\leqslant5$ min).

Figure 1*b* shows the variation of refractive index $n(D)$ with surface separation D for PEO1. These values of $n(D)$ were measured both in compression and decompression, and were the same (within error) both in solution and following the replacement of solution by pure electrolyte, at the two incubation concentrations. They indicate (as for polystyrene adsorbed onto mica from cyclohexane) that there is little desorption or extrusion of polymers over the times and in the conditions of our experiments. The adsorbance Γ of polymer onto the mica may be estimated from $n(D)$ (ref. 1), and corresponds to $\Gamma\simeq5$ mg m^{-2} of mica surface. To our knowledge there are no independent measurements of PEO adsorbance onto plane surfaces to compare this value of Γ with, though we note that it is comparable to adsorbance values for other polymers on plane surfaces[5]. It is, however, higher than PEO adsorbance onto colloidal silica particles[6] (for which $\Gamma\sim1$ mg m^{-2}).

The onset of interactions between the adsorbed layers in the present study suggests an effective extension of polymer from each mica surface of some $3R_g$. This compares with values of $\sim2R_g$ measured for various polymers (but not PEO) adsorbed on plane surface from θ-solvents[7], and with an effective extension of some $1.5\,R_g$ for polystyrene adsorbed onto mica in worse-than θ conditions[1]. Our value of 2.5–$3R_g$ is comparable with a hydrodynamic thickness of about $2R_g$ for PEO layers adsorbed onto polystyrene latex particles in a good aqueous solvent, as measured by light scattering[8], and also with theoretically predicted values of some $3R_g$ for adsorbed poly-

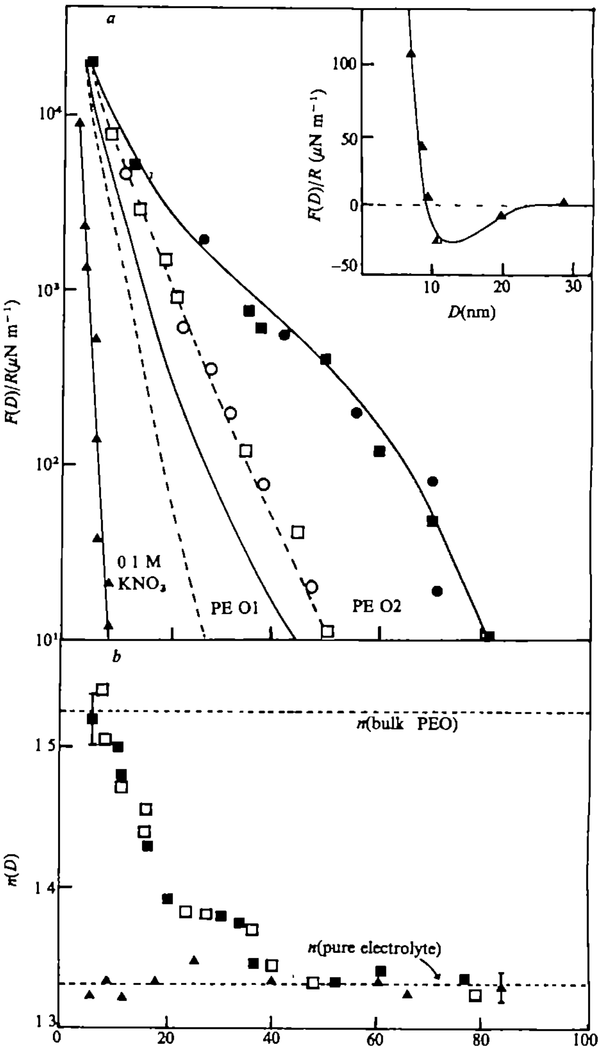

Fig. 1 *a*, Forces $F(D)$ between curved mica surface (mean radius of curvature $R\simeq0.5\times10^{-2}$ m) in 0.1 M KNO$_3$ (BDH Analar grade, in de-ionized double-distilled water) at 20±2 °C. ▲, Force profile in pure electrolyte. The inset shows the secondary minimum on a linear scale. PEO2: Forces $F(D)$ following 16±2 h immersion in 1.5×10^{-4} g ml^{-1} PEO2 solution. ■, Compression of surfaces following prolonged (>60 min) separation. □, Compression ~5 min after rapid decompression of surfaces. ●, Slow decompression. ○, Rapid decompression. Intermediate rates fall in shaded region. See text for details. PEO1: Forces $F(D)$ following 16±2 h immersion in PEO1 solutions. Solid line, compression after prolonged (>60 min) separations; also slow decompression. Dashed line, rapid decompression. Both the pure electrolyte and the polymer solutions were filtered (0.22 μm Millipore) before use. *b*, Mean refractive index $n(D)$ of medium separating mica surfaces. ▲, in 0.1 M KNO$_3$; ■, following 16±2 h incubation in 10^{-5} g ml^{-1} PEO1 solution; □, following incubation in PEO1 solution and the subsequent replacement of the solution by pure electrolyte. Values unchanged over 24 h.

mers in an athermal solvent[2]. The monotonically repulsive nature of the interactions suggests that for this system there is little effect of bridging or desorption (which could lead to attraction)[2,9], the forces being due to an increasingly repulsive osmotic interaction as the opposing polymer layers come into overlap in the good solvent[2,3,9].

Our results contrast with the earlier study by Israelachvili and co-workers, who used a similar method to measure $F(D)$ between mica surfaces in an aqueous solution of highly polydisperse PEO[10] (Carbowax resin WSR N80, Union Carbide). Their investigation indicated the continuous buildup with time of progressively thicker layers of surface adsorbed material (up to 1,000 nm over 24 h), as well as complex short- and long-time hysteretic behaviour suggestive of gel-like material on the surfaces. This may be due to the polydisperse nature of the polymer, as well as the possible presence of microgels or trace chemical impurities in the as-received Carbowax Resin (ref. 11 and J. N. Israelachvili, personal communication).

The present findings indicate that the interactions between adsorbed layers of monodispersed PEO across a good aqueous solvent are monotonically repulsive, and may be represented by a quasi-equilibrium force–distance profile at times longer than ~1 h. The extension of the adsorbed layers from each surface is somewhat larger than corresponding, independently measured values for polymers on plane surfaces in θ-solvents; the refractive index data indicates (in accordance with previous studies) a quasi-irreversible adsorption of polymer onto the mica surface.

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Magnetostratigraphical dating of loess deposits in China

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The age of Chinese loess deposits has long been disputed. Biostratigraphical and earlier magnetostratigraphical investigations placed the entire loess formation within the Pleistocene. The new palaeomagnetic measurements reported here on a bore hole section near Lochuan (Shaanxi province) suggest a clearly defined magnetic polarity zonation which extends below the Olduvai subchron. A late Pliocene age of ~2.4 Myr is assigned to the oldest loess sediments measured. The intensity variations of natural remanent magnetization (NRM) and low field magnetic susceptibility are indicative of climatic changes during loess deposition.

In northern China nearly half a million square kilometres are covered by loess sediments. Along the middle Huangho valley these wind-transported, yellow to red-brown sediments occupy a coherent area of more than 300,000 km^2, known as the loess plateau[1]. Here the loess deposits generally approach a thickness of 80–120 m. Typically they consist of an alternation of (1) silty loess layers which developed in cold and arid climatic conditions and (2) clayey loess or palaeosoil beds which are indicative of warm and humid climate (see Fig. 1: succession of loess- and soil(S)-horizons). Some of the soil horizons are developed in such a way that they can be recognized and correlated over large distances. They serve as stratigraphical marker horizons[2]. The environmental variations during loess formation are documented by faunal changes, especially of snail assemblages[3], by the degree of crystallization of clay minerals[4], and by carbonate content and other geochemical indicators[5].

On the basis of lithology, unconformable contacts and erosion surfaces the loess deposit has been subdivided into three main stratigraphical units[6–9] which all have been assigned a Pleistocene age: The Malan Loess of late Pleistocene age, The middle Pleistocene Lishih Loess and the early Pleistocene Wucheng Loess (Fig. 1). The biostratigraphical age estimates have been supported by several palaeomagnetic investigations[10–12]. According to remanent magnetization directions obtained after alternating field (a.f.) demagnetization, the Brunhes–Matuyama boundary has been found to coincide roughly with the Wucheng–Lishih transition. The Jaramillo subchron was identified in the middle of the Wucheng division, thus the oldest strata were estimated to have an age of <1.2 Myr.

We have sampled a long core from a bore hole near Lochuan (Shaanxi province) ~800 km south-west of Beijing (lat. 35.4 °N; long. 109.5 °E) for palaeomagnetic analysis. The core penetrates a 136-m thick loess sequence which has been deposited on top of an Upper Pliocene (Hipparion bearing) lateritic substratum. More than 230 samples were obtained from the core with an average sampling interval of 60 cm. The samples were oriented relative to the vertical but not azimuthally. Before demagnetization the NRM intensity is relatively strong with a mean value of 5.08×10^{-2} Am^{-1} for 150 soil samples, and 2.26×10^{-2} Am^{-1} for 181 loess samples. The low field susceptibility of the soil horizons (mean: 2.49×10^{-3} SI units) also is about twice that of the loess layers (mean: 1.16×10^{-3} SI units). High field experiments show that the main magnetic minerals are magnetite and haematite. The NRM is strongly affected by secondary, viscous remanent magnetization (VRM) so that the NRM vector always gives inclinations consistent with the present field of the Earth. Advantage was taken of this property to detect several misoriented (inverted) samples. Because major portions of viscous magnetization (up to 80% of total NRM) are removed by low a.f.s of the order of 10–15 mT, the VRM apparently resides mainly in magnetite. However, viscosity tests in the laboratory show that progressive a.f. demagnetization increases the instability of NRM. A.f. cleaning of NRM often results in rather erratic or badly defined demagnetization curves. Thermal demagnetization also removes a large secondary VRM component up to 200 °C, but above 300 °C a well defined and stable component is observed which has either positive or negative inclination depending on the stratigraphical position. On orthogonal vector plots this characteristic component decreases undirectionally towards the origin of projection on further heating. Maximum unblocking temperatures are usually above 575 °C. Thus the characteristic remanent magnetization of the loess is probably carried by haematite. Details of the loess rock magnetic properties will be described elsewhere.

After thermal demagnetization at 350 °C the core is subdivided into several clearly defined polarity zones, separated by sharp transitions (Fig. 1). In contrast to the results of earlier investigations which were based on a.f. cleaned NRM directions[10–12], the positive and negative inclinations average vectorially to the absolute value of the present local geomagnetic field inclination ($I = 52.5°$). From top to bottom of the core the polarity changes are correlated with: (1) the Brunhes–Matuyama boundary at core depth 53.05 m; (2) the Jaramillo subchron between 67.30 and 72.50 m; (3) the Olduvai subchron between 107.40 and 113.10 m.

No definite evidence of shorter term geomagnetic excursions is indicated in our data. Three samples (out of a total of 231)

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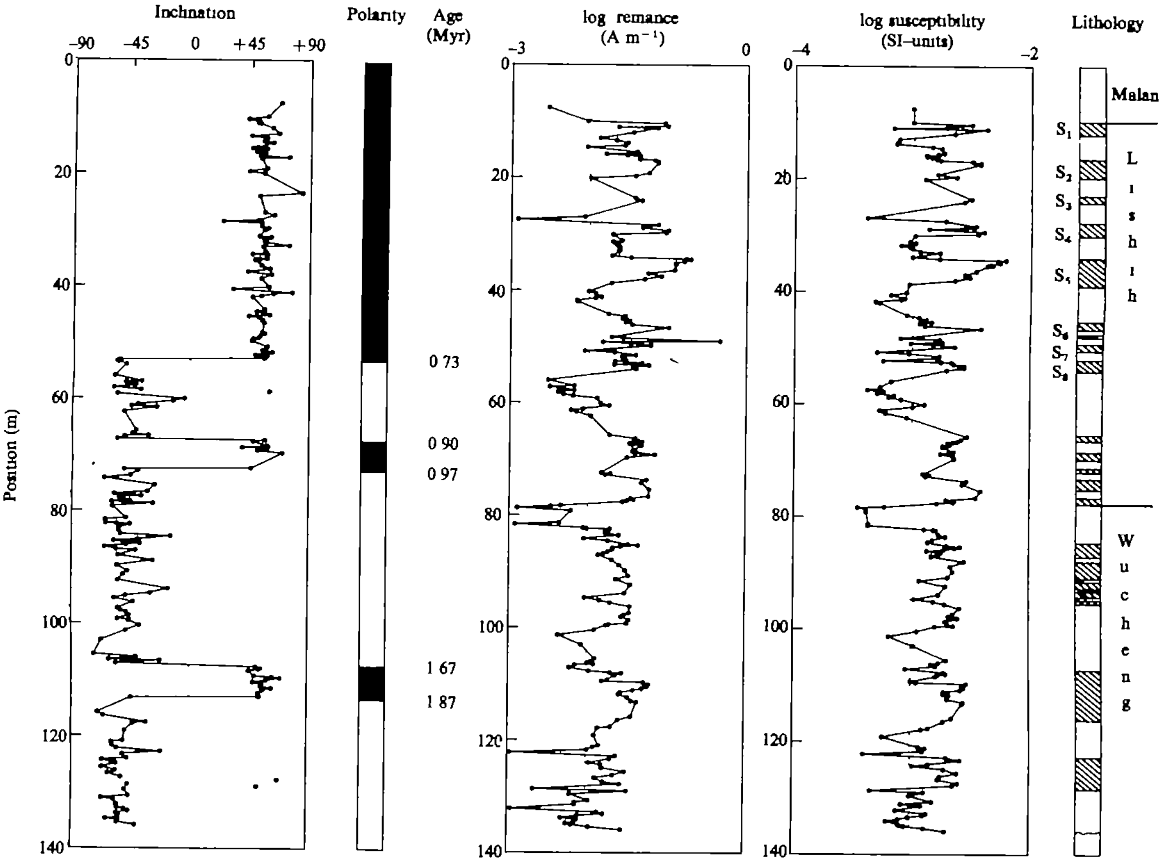

Fig. 1 Magnetic properties of the Lochuan bore hole versus core depth. Inclination data were mostly cleaned at 350 °C. The ages of polarity chron boundaries are according to Mankinen and Dalrymple[13]. Remanence intensities and susceptibilities are plotted before thermal cleaning. Lithological sequence of loess (white) and numbered soil layers (hatched) is according to Liu *et al.*[2].

do not fit to the observed polarity sequence. Possibly these samples may be completely remagnetized by a normal (present day) field or may suffer from unrecognized misorientation. Alternatively, they may indeed record field excursions or short polarity chrons (the two samples near 130 m depth would fit closely the Reunion events). Viscous remagnetization still affects the data to some extent, since the reversely magnetized portions of the core show higher directional scatter. No correlation is found between polarity and remanence intensity (Fig. 1) or low field susceptibility.

Our observations require a revision of some earlier ideas about the dating of Chinese loess. The Brunhes–Matuyama boundary is found to occur much higher in the loess profiles than thought previously[10–12]. The Olduvai event has been identified for the first time, implying much greater ages for the entire loess formation. Converting depth into age by using the time scale of Mankinen and Dalrymple[13] we notice an increase in apparent accumulation rate from 4.6 cm kyr^{-1} below the Jaramillo event to 7.3 cm kyr^{-1} in the upper 73 m. By linear extrapolation beyond the Olduvai event and due to the fact that the Matuyama–Gauss boundary is not observed in the loess sediments, we assign a minimum age of ~2.4 Myr to the beginning of loess sedimentation. This date coincides with age estimates for a general deterioration of climate in the Northern Hemisphere[14]. The age of the Plio–Pleistocene boundary is still controversial; however, if it were correlated with the top of the Olduvai event[15,16], then Chinese loess deposition, the older portions of which cover nearly the complete Matuyama epoch, started before the Pleistocene and dates back into the late Pliocene like the Central European loess deposits[17]. Also, the stratigraphical boundary between Lishih and Wucheng Loess no longer coincides with the Brunhes–Matuyama boundary, but has to be placed at ~1.1 Myr BP as it lies below the Jaramillo. Note that the negative polarity recorded in the bottom part of the loess profile (Fig. 1) continues down into the top of the Upper Pliocene red clay. After a few metres the polarity in the clay reverses again to normal (not illustrated in Fig. 1). As the lithology changes from clay to loess soil rather gradually, it is tempting to assume continuous sedimentation between clay and loess so that this reversal would actually document the Matuyama–Gauss boundary.

The intensities of NRM (before cleaning) and low field susceptibility (Fig. 1) vary by 2–3 orders of magnitude throughout the section, being closely correlated to each other. High intensities characterize the soil horizons, whereas low values are typical of loess deposits[11]. These features are ascribed to the pedogenetic processes of rock formation which favour haematite enrichment in the soils due to alteration in a relatively warm and humid climate. The carbonate content of the soil horizons is reduced[2] causing an overall volume decrease of up to 20%. Pre-existing magnetite becomes oxidized, the iron available from other minerals is mobilized and re-precipitates later in the form of oxides and hydroxides[18,19]. Especially the latter process of formation of ferromagnetic minerals is thought to cause the intensity fluctuations of remanence and susceptibility which therefore are due to climatic changes and not to geomagnetic field variations as suggested by Wollin *et al.*[20] for Pleistocene deep sea sediments.

Thus the stable part of NRM may have a completely chemical origin. Using the magnetic intensity record we can estimate that about 17 glaciations have occurred in China since the Olduvai event. This number of glaciations is similar to that encountered in European loess deposits[17,21]. It is also consistent with the number of oxygen isotope stages in post-Olduvai deep sea sediment from the Atlantic[22]. The Brunhes–Matuyama boundary is found in the soil layer S_8 (Fig. 1) which follows a loess unit deposited during a major glaciation period. Similar observations have been made in the oxygen isotope record of the deep sea sediments where this field polarity change is placed within the interglacial stage 19 (refs 22–24). The rock magnetic intensity variations become especially pronounced after 1.2 Myr ago (above core depth 82 m) and are interpreted to record rather drastic climatic changes coinciding with major episodes of loess deposition at about 1.15, 0.85, 0.55, 0.44 and 0.18 Myr ago (age estimates by linear interpolation). The first of these episodes, presumably marking major glaciations, is accompanied by an increase of sedimentation rate by about a factor of two.

Our investigation demonstrates that, after thermal demagnetization, the NRM of the loess deposits in China renders a reliable record of geomagnetic field polarity. The rock magnetic properties apparently are largely controlled by chemical alteration processes during rock formation and therefore reflect climatic fluctuations during late Pliocene to Pleistocene times.

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Origin of desert loess from some experimental observations

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A longstanding problem of Quaternary geology has been the origin of the silty deposit known as loess. Of the many explanations for its formation and distribution[1,2], the most commonly accepted is that the silt is produced by subglacial grinding processes[1,3,4], followed by redistribution and deposition by the wind. The large expanses of loess in central Europe are therefore considered to be the consequence of Quaternary glaciation[5]. There is, however, a view[6,7] that loessic silt may also originate as the product of processes in hot deserts. Some proponents of the desert loess hypothesis consider that silt-size material is the product of weathering processes[7] which may include salt weathering[8] or weathering down incipient cracks[9]. An alternative mechanism is aeolian attrition of sand grains, although, probably as a result of the experimental work of Kuenen[10], Smalley and Krinsley[11] consider that only fine silt (2–6 μm) is produced in large quantities by the abrasion of quartz grains. We have simulated aeolian attrition of angular quartz grains previously produced by weathering in deserts. The products of abrasion show that both coarse and fine silt sizes are produced. These findings suggest that desert aeolian processes can produce loess. We also suggest that much of this material from many deserts has been deposited in the sea but that the Chinese loess could have been produced in the Gobi desert. The finest particles produced by such attrition may be a source of silica for silcrete formation.

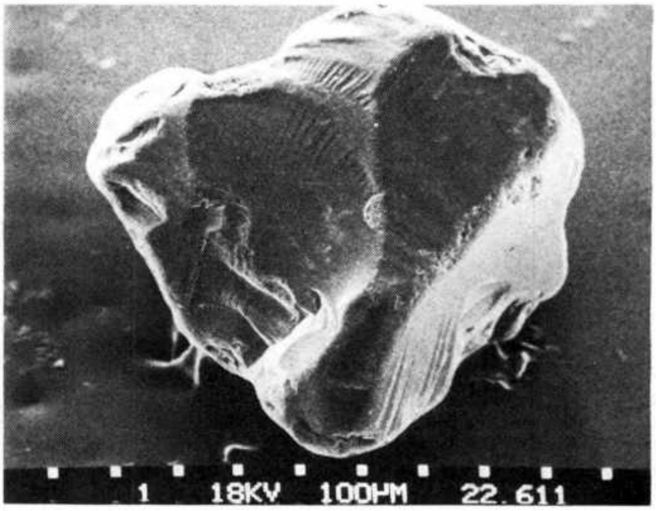

Fig. 1 An originally angular grain after 600 h of gentle erosion in the attrition device. At this stage the abrasion is confined to edges and corners but has already produced a considerable quantity of fragments. On this, and subsequent micrographs, the scale is given by the interval between the successive white squares at the bottom; the value (in μm) the second group of figures from the right.

We have concentrated on the aeolian attrition of quartz sand size grains released from the parent rock by weathering. In particular, whether there is a mechanism to produce particles in the 20–50 μm range from coarser material. Previous experiments on sand abrasion[10,12] have tended to examine the larger products of attrition without either collecting or examining the smaller fragments chipped off the sand size grains. These fragments may, however, provide the very silt size material that the experiments were designed to detect. We have designed an experimental apparatus which, we believe, samples all the products of any attrition taking place. A triple-head 500-ml flask was used as an abrasion chamber in which ~10 g of crushed vein quartz (710–840 μm sieve size) was placed and agitated gently in a regulated airstream. The centre orifice was used to introduce a glass tube which vented air just above the base of the flask. The grains followed a regular saltating path with both interparticle collision and dune face avalanching. The 'air velocity' means little in these conditions but as field conditions are themselves very variable we do not consider this a limitation. Sampling of attrition debris was carried out in the outflow air at the top of two 300 mm high 'stilling wells' positioned on the flask side necks. This arrangement prevented the main, original particles from escaping. A scanning electron microscope (SEM) stub was placed in this airstream with double-stick tape on its surface. This was changed at intervals to allow periodic examination of small particles. The exhaust from the stilling wells was allowed to escape down polyethylene tubing. Fine particles are trapped by their own electrostatic charge in the first 10–20 mm and this region was removed at each sampling interval. The tube was cut and a portion examined by use of the SEM, together with fragments caught on the stub and 10 of the original grains in the flask. Collection efficiency is believed to be better than 95% of the fines produced. We have been able therefore to examine not only the abrading, original, grains and their progressive attrition but also the nature of the fine particles produced. Vein quartz, without apparent weaknesses introduced by the crushing, was used because the experiment was designed to look at the products of attrition as far as possible. Kuenen's experiment also used this type of material.

Figure 1 shows a grain after 600 h of gentle abrasion. Although it is rather poorly rounded compared with Fig. 2 the latter is from a desert dune environment[13] and has been abraded for a very much longer period and probably at much higher tractive velocities. Indeed, it is surprising that such abrasion

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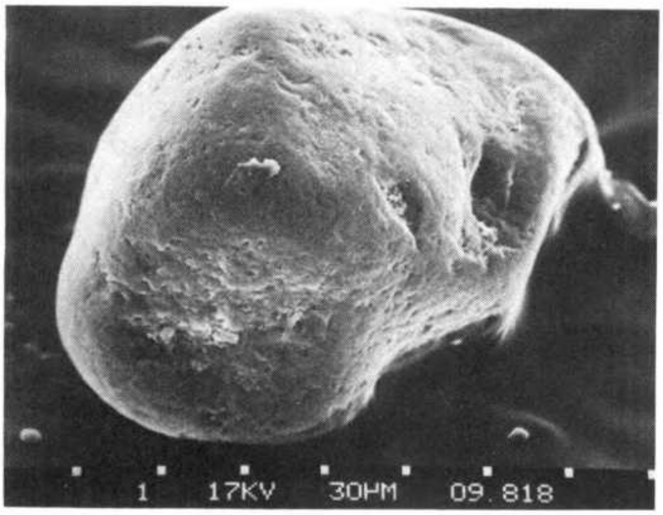

Fig. 2 This grain was taken from a fossil desert dune environment in Nigeria. There is considerably more rounding than seen in Fig. 1, abrasion now covers faces rather than just on corners. It is not known how long this grain might have been subjected to abrasive action but is likely to be very much longer than 600 h. The deposits are probably at least 25,000-yr old but the length of active dune formation is not known.

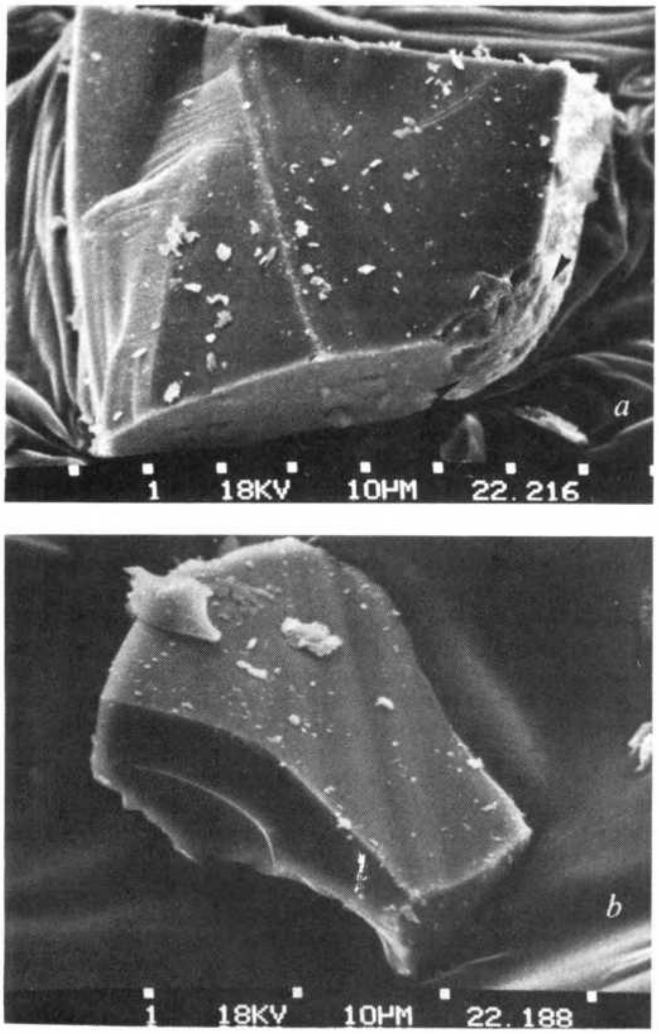

Fig. 3 Two coarse silt fragments (at roughly the extremes of this size range) produced by the attrition in the experiments reported. *a*, A grain with a section which has been rounded (between the arrows). The surface texture of this area is similar to that seen in Figs 1 and 2. *b*, A finer fragment with no such surface texture apparent. *a* was extracted after 200 h of attrition and *b* after 60 h.

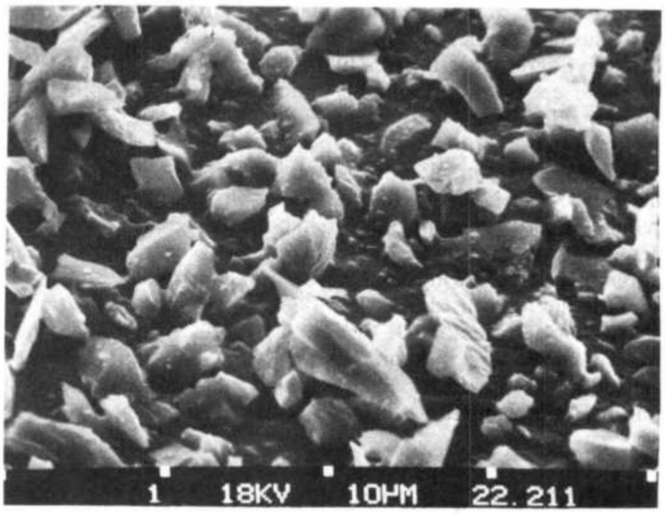

Fig. 4 Finer material than that seen in Fig. 3 is produced in the experiment. This micrograph shows fine silt and clay-size material caught in the polyethylene final trap.

seen in Fig. 1 has occurred. Not all desert grains are as rounded as that in Fig. 2 (ref. 14), this is possibly because they have not been abraded either for very long or sufficiently energetically. The abrasion process is one of progressive removal of small fragments, generally <30 µm but with rather fewer larger chips (<100 µm). Figure 3 shows two of these larger fragments which, in this case, are products of edge rounding. Such rounding, however, is not often visible. Chips of this size may be produced in the manner suggested by Moss[15] and Smalley and Vita-Finzi[4] but are rather less common than these investigators imply. Kuenen's[10] finding that "no quartz particles are produced in the size range dominating in loess" (although he did obtain some dust <5 µm) may have been a major factor in the reluctance to accept aeolian abrasion as a means of producing loessic silt. Our experiments have shown that silt-size as well as some clay-size material is formed in low energy aeolian environments (Fig. 4). Exact determinations of the quantities of attrition products is not possible but (after 60 h) a mass reduction of about 1–5% was found; of this about 20% was <5 µm. It is not yet known what are the relationships between particle velocity and size and quantity of fine material produced by aeolian action. In our experiment, fine material was still being produced after 600 h action. It is not known why Kuenen only detected the fine dust in his experiment. The surface textures seen in Figs 1 and 3*a* are very similar to that of the known aeolian origin of Fig. 2 and it seems reasonable to assume that the experimental production of fine material was matched naturally to give the rounded grain of Fig. 2.

There are several points of note. (1) Abrasion takes place primarily by point impacts of (originally) angular edges (Fig. 1) probably by grain spinning[16]. (2) Chips knocked off are of predominantly silt-size and are rather blocky in nature. This compares with published size ranges and SEM micrographs of loess[17–19]. (3) Abrasion may be achieved at very low particle velocities. (4) There is some resemblance of rounded edges to so-called 'chemical precipitation' surface textures seen on SEM micrographs of quartz grains from desert dunes[20]. (5) The very fine material produced in nature may be entrained by wind and removed completely from any desert system. The very finest abrasion products may even be dissolved and could be an important contributor to silicification of sediments[21].

Finally, it seems that an aeolian abrasional origin for some loessic material is quite possible. It is unlikely that much European loess is produced in this way even though long distance travel from the Sahara is possible[22]. Most Saharan produced silt lies not on the southeastern Mediterranean land surface[6,7] out in the Atlantic Ocean[23,24] or even South America in the case of fine dust[25].

It would, for example, seem that a mechanism may exist whereby weathered grains in the Gobi desert could produce a substantial amount of China's loess. It is also possible that silts in the south-west of the United States and northern Mexico were produced by an aeolian abrasion mechanism, perhaps coupled with silt production by weathering mechanisms[9].

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Trapped methyl radicals in chert

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We have demonstrated recently that electron spin resonance (ESR) spectroscopy can detect a narrow signal from electron gain (E′) centres in flint (Upper Cretaceous lepispheric chert)[1]. This signal, caused by the effect of natural radiation, is lost on heating and may be useful for dating[2]. ESR signals from various organic radicals are now reported in a wide variety of cherts. The thermal dependence of the signals may provide valuable information about the duration and temperature of ancient chert heatings.

The organic radical signals have been observed in Upper Cretaceous lepispheric cherts from Europe and the Middle East, and in Tertiary chalcedonic cherts from south-west France. The chalcedonic cherts comprise a coarse, fibrous α-quartz (chalcedony) and the lepispheric cherts predominantly comprise α-quartz lepisheres cemented by an interstitial microchalcedony.

The most important, and most unexpected result is the detection at ambient temperature of a well-defined quartet of lines (1:3:3:1) with a hyperfine coupling of 22.7G and a g-value of 2.0025 which is characteristic of methyl radicals[3] (See Fig. 1 and Table 1). In addition a triplet due to two equivalent protons has been detected which we assign to $X–\dot{C}H_2$ radicals. Other unassigned features due to organic radicals have also been detected.

Most of the cherts studied have given a very weak methyl signal. After heating the chert, the signal is more intense, the methyl concentration reaching a maximum at around 350 °C before rapidly decreasing (Fig. 2). The temperature giving maximum methyl radical concentration varies with the duration of heating. Surprisingly, γ irradiation of chert did not alter the methyl radical concentration. γ irradiation followed by heating gave less growth of the methyl signal than did an identical heating of an unirradiated sample. It is thus necessary to postulate a radiation sensitive precursor ($Y–CH_3$) which is thermally labile:

Table 1 ESR data for methyl radicals in various environments, together with data for $X–\dot{C}H_2$ species

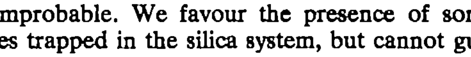
Radical	System temperature	g-value (average)	Hyperfine coupling (G)	Ref.
$\cdot CH_3$	CH_3I/vycor glass (RT)	—	22.7	4
$\cdot CH_3$	CH_4 (4K)	2.0025	22.9	5
$\cdot CH_3$	Chert (RT)	2.0025	22.7	This work
$\geqslant SiO\dot{C}H_2$	CH_3OH/vycor glass (−140 °C)	2.0031	20.5	6
$X–\dot{C}H_2$	Chert (RT)	2.0031	21.0	This work

$1G = 10^{-4}$ T; RT = room temperature

$$Y–CH_3 \rightarrow Y\cdot + \cdot CH_3$$

Several aspects of the environment of these $\cdot CH_3$ radicals can be delineated. The fact that the lines are narrow shows that the radicals are freely rotating, but not in the 'gas-phase'[3]. Also, the absence of 'spin-flip' satellite features at high power indicates that they are trapped in an environment free of paramagnetic nuclei[3]. Obviously, they cannot be close to any species with which they can react, and they are well separated from the radicals Y· (if they are radicals) because there is no spin–spin reaction broadening the lines. Almost certainly they are trapped in molecular-sized holes in a purely silica environment.

Similar comments apply to the $X–\dot{C}H_2$ radical. Furthermore, the group X- must contain only non-magnetic nuclei close to carbon as no further hyperfine coupling can be detected. γ irradiation increases the concentration of $X–\dot{C}H_2$ radicals. Their thermal behaviour is complex. The precursor of the methyl radicals, $Y–CH_3$, is unknown. Species such as



$$\begin{matrix}—O\\—O\\—O\end{matrix}\!\!>\!Si—CH_3 \quad \text{or} \quad \begin{matrix}—O\\—O\\—O\end{matrix}\!\!>\!Si—O—CH_3,$$

though attractive, are unlikely as there is no reason to believe that the Si—C or O—C bonds are exceptionally weak. These bonds might be weakened by the proximity of a radical centre, but since the thermal yield of methyl is decreased by irradiation this is improbable. We favour the presence of some labile molecules trapped in the silica system, but cannot guess their identity at this stage. It is possible that the same species is the source of $X–\dot{C}H_2$ radicals on radiolysis (that is, $X \equiv Y$), as this

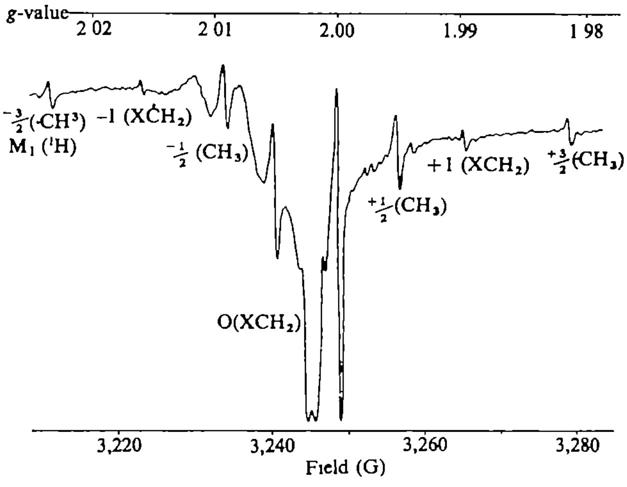


Fig. 1 Electron spin resonance spectrum of a chert sample from Ksar-Akil, Iraq, recorded at room temperature showing signals assigned to $\cdot CH_3$ and $X–\dot{C}H_2$ radicals. The sample volume was $\sim 0.2\ cm^3$. Spectra were recorded on a Varian E109 spectrometer capable of detecting $\sim 10^{11}$ spins.

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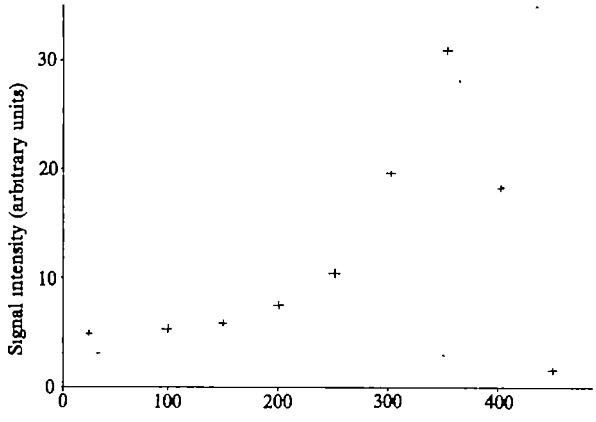

Fig. 2 Isochronal annealing curve showing the intensity of the methyl signal (measured at room temperature) after successive heating episodes of 15 min duration at each of the marked temperatures. The chert sample was from Al-Beidah, Jordan.

would account for the lower yield of methyl radicals on heating after irradiation. It seems to be important that many varieties of chert should contain a compound that is a thermal source of methyl radicals, since such compounds are most unusual.

The thermal behaviour of the organic radicals in chert is being further studied and is likely to prove useful in determining the temperature and duration of ancient heating episodes. Such information is of considerable interest in the study of ancient stone-working techniques. It is also useful in determining whether, or not a sample has been sufficiently heated to be suitable for dating by thermoluminescence of ESR techniques.

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Effects of aerosols on photosynthesis

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Air pollution can cause various problems to agriculture through its direct or indirect effects on plants[1,2]. The net effects of air pollution on agricultural crop yields are very difficult to estimate quantitatively, and are generally considered secondary to the effects of fertilizers or chemical disease control measures. However, continually increasing air pollution may represent a persistent and largely irreversible threat to agriculture in the future. Here we have examined only one indirect aerosol effect on agricultural plant life—the reduction in photosynthetically active radiation (PAR) reaching the ground in the presence of significant air pollution. This effect can be quantified confidently from results of detailed model calculations of the transfer of solar radiation through the atmosphere with varying levels of air pollution. For the biologically effective wavelength region between 0.35 and 0.72 μm, the reductions in PAR due to increasing tropospheric aerosol loads have been determined for rural and urban pollution scenarios. For example, for the north-eastern United States during the summer months, a reduction in PAR of about 20% and 33% was obtained when the visual range due to rural and urban aerosols, respectively, was decreased from clear air conditions to 10 km.

The effects of aerosols on the natural radiation balance in the atmosphere have been studied by using extensive measurement programmes[3] and theoretical modelling[4]. Only recently, however, have detailed data on realistic air pollution scenarios become available to allow an accurate description of the optical parameters of aerosol-loaded atmospheres. For our model calculations we used the very detailed and recent data base for the optical properties of atmospheric aerosols compiled by the Air Force Geophysics Laboratory (AFGL)[5,6]. Four altitude regimes with different aerosol models in each region are considered. Within the boundary layer (up to 2 km height), rural and urban-type aerosols are specified with varying meteorological ranges (surface visibilities). These models are intended to distinguish between aerosol types of natural and man-made origin over a land area. The 'rural model' represents aerosol conditions over continental areas having natural environments that are not directly influenced by anthropogenic pollution. These aerosols are assumed to consist of a mixture of 70% water-soluble substances (ammonium and calcium sulphate and also organic components) and 30% dust-like aerosols. The refractive index for the individual components is based on the measurements of Volz[7]. The size distribution is parameterized as the sum of two log-normal size distributions. In contrast, the 'urban aerosols' are considered typical to stagnant polluted air originating from combustion products and industrial sources. This model, therefore, is taken to be a mixture of 80% rural aerosols with 20% carbonaceous aerosols. These soot-like aerosols are assumed to have the same size distribution as both components of the rural model.

To describe aerosol attenuation in finite wavebands, integral turbidity coefficients are often used[8]. For simplicity, we characterize an existing pollution level by its associated surface visibility. Using Koschmieder's law, the visual (meteorological) range V(km) is inversely proportional to the total extinction coefficient at 0.55 μm: $V=3.91/\sigma$, where $\sigma=\sigma_m+\sigma_a$ (km^{-1}) ('m' and 'a' refer to molecules and aerosols, respectively). In our data base, clear air (that is, $\sigma_a=0$), corresponds to $V=$ 300 km. For increasing pollution levels, the visual range decreases from 300 to 2 km as the aerosol concentration increases from zero to 70,000 particles cm^{-3} for urban aerosols (86,000 particles cm^{-3} for rural aerosols) in the boundary layer up to 2 km height. The particle number densities depend strongly on the relative humidity and the particle size distribution[5]. The values given are for a relative humidity of 90%. In the rest of the atmosphere (up to 70 km height) a constant (light) background pollution level is assumed: the 'tropospheric' aerosol model[5] up to the tropopause and the 'background stratosphere' model[5] above the tropopause. This background aerosol is considered to consist largely of sulphate particles formed by photochemical reactions. Aerosol angular scattering is represented by a height-dependent Henyey–Greenstein phase function:

$$P(\theta, z)=\omega_0(z)[1-g^2(z)][1-g^2(z)-2g(z)\cos\theta]^{-3/2} \quad (1)$$

where ω_0 is the aerosol single scattering albedo, and g is the asymmetry parameter. The total phase function is the appropriately weighted average of Rayleigh (molecular) scattering and aerosol scattering as a function of height, z.

Our new calculations use a highly efficient and versatile radiative transfer code[9] in which we incorporate the detailed atmospheric data base described above[10]. The computational method is based on the finite-element discrete-ordinates solution of the radiative transfer equation and has been verified[11] against other calculations and actual telephotometer measurements. The computations include the anisotropic scattering characteristics of aerosols, according to equation (1), as well as the detailed vertical distribution profiles for aerosols, molecular scatterers (mainly Rayleigh scattering) and absorbers, as described in the AFGL data. All orders of scattering are taken into account.

We compute the total solar irradiance at ground level for 42 wavelength bins in the photosynthetically active regime from

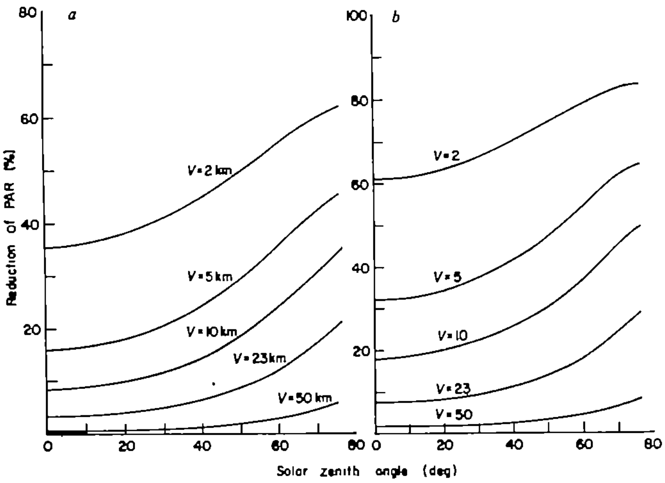

Fig. 1 Per cent reduction of photosynthetically active radiation (PAR) for corn plotted against solar zenith angle. Degree of air pollution is indicated by the surface visual range, *V*. *a*, Mid-latitude summer atmosphere with rural aerosols in boundary layer. *b*, Mid-latitude summer atmosphere with urban aerosols in boundary layer.

0.35 to 0.72 μm assuming varying aerosol concentrations in the 2 km surface boundary layer. A measure of the biologically effective irradiance is the PAR, defined by

$$\mathrm{PAR} = \int_{\lambda_1}^{\lambda_2} P(\lambda) I(\lambda, Z_0)\,\mathrm{d}\lambda \qquad (2)$$

where $I(\lambda, Z_0)$ is the spectral irradiance at ground level for the solar zenith angle Z_0, and $P(\lambda)$ denotes the photosynthesis action spectrum for a particular plant. Equation (2) broadens the conventional definition of PAR by introducing the weighting function $P(\lambda)$, a convenient way of characterizing the special response of a specific plant species; λ_1 and λ_2 are the wavelengths for which $P(\lambda)$ tends to zero. Photosynthesis action spectra measured for 22 crop plants[12,13] were found to be fairly similar. Therefore, we report here only our results for corn, which is typical of most agricultural crop plants.

Figure 1 shows the percentage reduction of PAR compared with clear air for five different surface visual ranges that arise when the amount of rural or urban aerosols in the 2 km boundary layer is increased. The increasing values for all curves with increasing solar zenith angle are clearly due to the increased path length for the solar radiation as it passes through the atmosphere at larger angles of incidence. Any fixed solar zenith angle may be thought of as an effective zenith angle, determined as a daily average over the continuously varying direction of the Sun at a given date and latitude[11]. For example, for the northeastern United States during the summer months, an effective solar zenith angle of 55° is appropriate. In this case we conclude from Fig. 1*a* that if the visual range due to rural aerosols decreases to 10 km, the PAR for corn will be reduced by about 20% compared with clear air conditions. For urban aerosols (Fig. 1*b*) the respective PAR reduction is 33% for the same visibility degradation. In the case of more severe pollution, the calculated PAR reduction increases nonlinearly to very high values, for example, for $V = 2$ km, urban aerosols and $Z_0 = 55°$, we obtain a 75% PAR reduction. As a typical example, a visibility of 20 km (10 km) and more has been observed[14] over the Chicago Metropolitan area for only 40% (70%) of the time between 1970 and 1972.

We note that in the solar radiation measurements of McCartney[15], only small turbidity changes ranging from 0.1 to 0.5 were found. However, when the visual range decreases from 50 to 5 km, the turbidity coefficient (average optical depth, τ) increases from 0.1 to 1.0. The intensity of direct sunlight, which is proportional to $\exp(-\tau)$, therefore decreases by about 60% while the diffuse radiation field due to multiple scattering increases.

The large calculated PAR reductions demonstrated here may appear alarming. Note, however, that any reduction in photosynthetically active radiation cannot be translated directly into an equivalent crop yield reduction because many additional effects in plant physiology and local climatology must be considered also.[16,17]. For example, the so-called C_3 plants reach saturation of their daily photosynthesis at radiation intensities of about one-third full sunlight. Other species, for example the C_4 plants like sugar cane and maize, increase their net photosynthesis continually up to very high irradiance. Also, if constant high air pollution levels are considered, shifts in the main growing season may occur that also affect plant growth and crop yield. Therefore, to estimate quantitatively the influences of our calculated PAR reductions on crop yield, an additional dynamics system study is required for which our model results could be used as input.

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No significant deviation from random mating of worldwide populations of *Drosophila melanogaster*

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Multiple choice experiments using 29 populations of *Drosophila melanogaster* from around the world were performed to measure any divergence in the mating system of this species. Courting flies were observed in a total of 691 mating experiments and 9,280 copulations. Only two of the 38 interpopulation crosses exhibited a significant divergence from random mating. However, neither of these represent a trend towards positive assortative mating. This suggests that there is considerable stability in the mate recognition system of *D. melanogaster* in spite of the substantial genetical and morphological differentiation which exists throughout the cosmopolitan distribution of this species.

Components of mating success in *Drosophila*. are under genetic control[1] and in laboratory populations the component of darwinian fitness which includes the 'selection' of mates is important in determining reproductive success[2–4]. Genetically based variation in 'premating isolating mechanisms' occurs among geographically separated populations of a species[5–7], either as a result of selection, genetic drift, or both[5,8,9]. Although much is known about the genetics of reproductive behaviour of *D.melanogaster* in the laboratory, studies have mainly used

Table 1 Results of multiple choice experiments among populations of *Drosophila melanogaster*

Cross	No. of expts	No. of matings ♂1×♀1	♂2×♀1	♂1×♀2	♂2×♀2	Pairs mated	% Mated	ZI s.e.	
23×28	18	61	48	75	64	248	62	1.04±0.14	
27×23	24	76	64	93	73	306	58	0.96±0.10	
28×27	23	78	73	85	73	309	61	0.96±0.10	
26×24	26	77	116	87	91	371	65	0.83±0.09	*8.9
28×26	18	49	54	67	69	239	60	0.97±0.13	
22×7	10	27	30	31	39	127	58	1.06±0.17	
27×9	18	53	52	61	66	232	59	1.05±0.14	
2×15	25	76	82	83	96	337	61	1.04±0.10	
3×2	18	71	67	58	53	249	64	0.98±0.12	
3×6	18	63	72	59	68	262	66	1.00±0.12	
2×28	18	53	56	54	56	218	55	0.99±0.14	
4×5	18	66	61	73	71	271	68	1.03±0.12	
22×3	18	63	62	56	49	230	58	0.94±0.14	
29×14	26	93	85	91	93	362	63	1.06±0.10	
3×10	18	64	82	63	68	277	70	0.91±0.13	
13×14	18	81	65	66	58	270	68	1.05±0.14	
23×19	22	85	87	72	85	329	68	1.07±0.10	
23×10	20	70	66	76	81	293	67	1.06±0.10	
19×9	22	87	66	78	62	293	60	1.02±0.12	
9×15	20	53	51	55	49	208	47	0.96±0.14	
8×13	20	56	69	58	70	253	58	1.00±0.12	
11×20	9	31	33	27	31	122	62	1.03±0.18	
11×12	11	32	36	36	39	143	59	0.98±0.17	
20×21	18	60	54	52	50	216	55	1.03±0.14	
25×17	20	63	59	70	71	263	60	1.04±0.12	
18×27	10	45	44	38	40	167	75	1.04±0.15	
22×15	10	32	38	34	32	136	62	0.89±0.16	
27×7	22	78	75	75	73	301	62	1.01±0.10	
4×14	15	37	37	53	61	188	57	1.07±0.16	*9.1
25×22	24	70	66	93	81	310	59	0.96±0.11	
15×16	17	52	43	55	53	203	54	1.07±0.15	
5×1	17	57	60	57	60	234	63	1.00±0.14	
9×10	22	77	75	73	74	299	62	1.02±0.10	
21×23	23	75	67	91	86	319	63	1.02±0.10	
13×6	22	62	52	78	65	257	53	1.00±0.12	
19×14	11	37	34	39	33	143	59	0.96±0.16	
12×21	11	39	39	35	36	149	62	1.01±0.16	
8×2	11	39	37	34	36	146	60	1.05±0.17	

The experiments were performed at 25 °C by direct observation, a technique originally devised by Elans and Wattiaux[10]. For each experiment 11 virgin males and females of one strain, plus 11 virgin males and females of a second strain, a total of 22 pairs, were used for every set of two strains. Fours types of mating could thus be observed:

♂♂1×♀♀1, ♂♂2×♀♀1, ♂♂1×♀♀2, ♂♂2×♀♀2.

Flies were kept separately for 5–7 days and then introduced into a mating chamber and observed at 6-min intervals for 1 hour. This duration allows for about 60% of the females to mate. Records were made of the number of within and between strain matings. In order to distinguish the flies of the different strains through the transparent cover of the observation chamber, a small dot was painted on the dorsal surface of the thorax with non-toxic ink (Rotring). This marker was rotated from strain to strain, so that no one strain in any combination of two strains tested together, always had the thorax painted. Painting was perfomed on lightly etherized flies at the same time as they were 'sexed' after emergence. Marking was found to have no effect on the strains studied here. All experiments were conduted between 0900 and 1200 hours. All experiments for any one cross were run on the same day. For analysis of the data, comparison with panmixia the χ^2 test was used. In addition isolation was measured by Levenes joint index[11,12]. The number of males and females of the two strains being equal, let the observed number of matings of the four possible types be given by:

	Strain 1 (♀♀)	Strain 2 (♀♀)
Strain 1 (♂♂)	X11	X12
Strain 2 (♂♂)	X21	X22

Levene defines the Joint isolation index as:

$$ZI=\sqrt{\frac{X11\cdot X22}{X12\cdot X21}}$$

ZI = 1 Means random mating. ZI > 1 Means a tendency to homogamic mating. ZI < 1 Means a tendency to heterogamic mating.

The exact distribution of these statistics is unknown but practical tests of significance can be based on the following estimates of variance:

$$\sigma^2(ZI)=\frac{(ZI)}{(4)}H \text{ where } H=\frac{1}{X11}+\frac{1}{X12}+\frac{1}{X21}+\frac{1}{X22}$$

* $P<0.05$, 3d.f.

mutant strains or well established wild-type cultures. We know surprisingly little about the genetic dynamics of the mating systems of natural populations. In order to test for any geographic differences in the male-female communication system, multiple choice experiments[10] were conducted among 29 populations derived from diverse localities around the world (Fig. 1). Wherever possible each population was derived from a large number of recently collected individuals, and populations were kept for only a short time before use. Thirty-eight crosses between localities were undertaken and results were tested for deviations from random mating using a chi-squared test (Table 1). This does not, however, indicate the degree and nature of the mating preference should a significant result appear and for this purpose the degree and nature of any mating divergence was measured using Levenes joint isolation index[11].

The data show no significant deviation from random mating. Only two crosses resulted in a significant deviation and neither of these are the result of a tendency to mate within populations. The cross between the two Russian populations 26 and 24, for example, is significant because males of population 24 have a higher propensity to mate. The remaining cross which yielded a significant result, cross 4 × 14, was the result of a higher mating propensity on the part of females of population 14.

Previous studies of natural populations of *D. melanogaster* involved crosses between French, Japanese and Afrotropical strains[12,13], also showed a lack of divergence. Our techniques were very similar so we propose that this is a general characteristic of populations of *D. melanogaster*. There is a similar lack of divergence in mating behaviour of populations of *D. pseudoobscura*, from western United States and Mexico[14]. The latter authors point out, however, that the opposite result is common in interpopulation crosses involving *Drosophila*

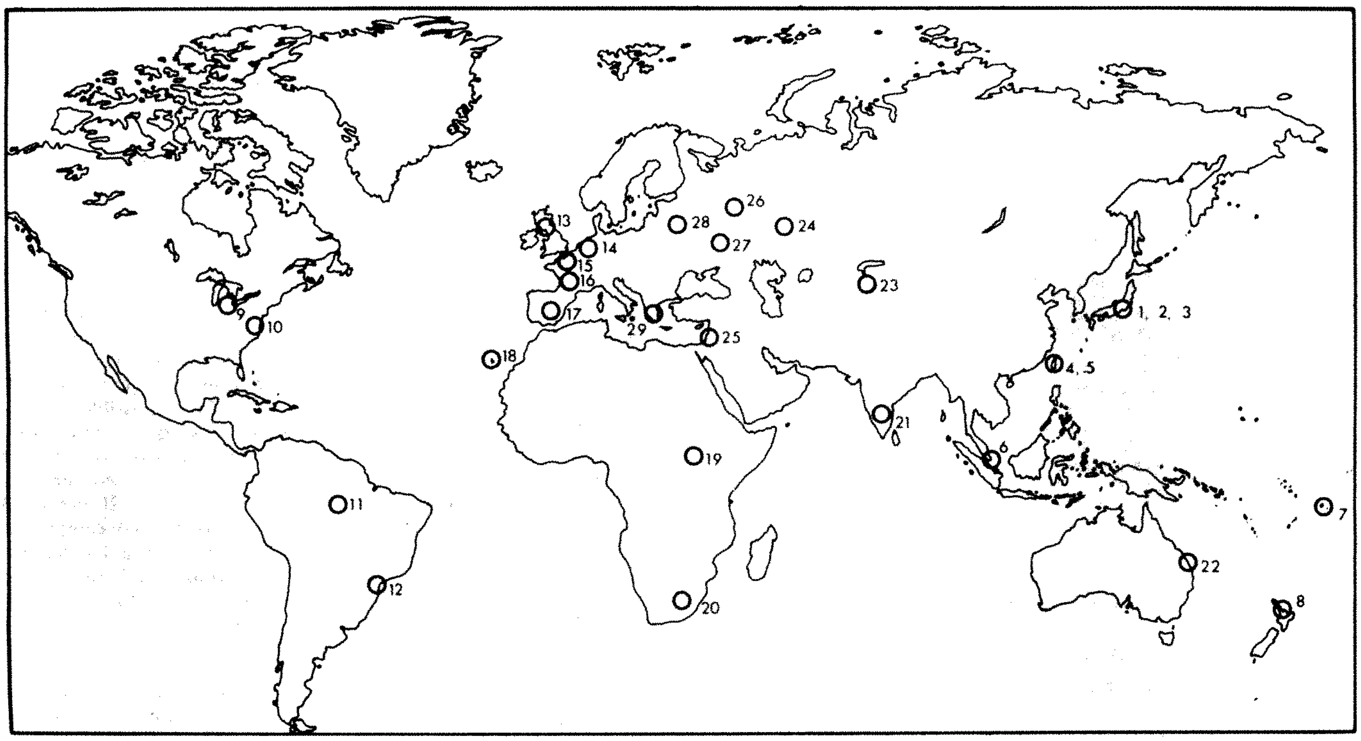

Fig. 1 Collection localities of strains of *D. melanogaster* used in the crossing experiments. The exact location, numbers of individuals from which the strains were derived, and date of collection are listed in parentheses. (1) Mishima, Japan; >10♀♀; August 1981; (2) Katsunuma, Japan; 50♀♀; October 1981; (3) Otsuki City, Japan; 8♀♀; September 1981; (4) Wulai, Taipei County, Taiwan; 1♀♀; November 1981; (5) Tong Pu, Nan-Tou County, Taiwan; 1♀♀; November 1981; (6) Singapore; large number of adults; 1979; (7) Suva, Fiji; 4♀♀; August 1981; (8) Auckland New Zealand; 20♀♀; February 1982; (9) Bowling Green, Ohio; large number of adults; October 1981; (10) Beltsville, Maryland; large number of adults; September 1981; (11) Jose Bonifacio (Sao Paulo) Brasil; 10♀♀ 8♂♂; September 1981; (12) Manaus (Amazonas) Brasil; 2♀♀ 3♂♂; November 1981; (13) Edinburgh; 20 adults; July 1981; (14) Gröningen, Holland; >30 adults; July 1981; (15) Ménétiéol (Sanciene) France; 28♀♀ 112♂♂; September 1981; (16) Iseré (Lyon) France; 200 adults; September 1981; (17) 70km from Madrid, Spain; 400 adults; November 1981; (18) Tenerife, Canary Islands; 25♀♀; September 1981; (19) Kampala, Uganda; 10♀♀; October 1981; (20) Pretoria, South Africa; 15♀♀; February 1982; (21) Nagarhoe, 15km Mysore, India; 11♀♀;February 1982; (22) Frazer Isle, Queensland, Australia; 50♀♀; September 1981; (23) Alma-Ata, Soviet Union; small number of adults; Mid-1981; (24) Birsk (50 miles from UFA); small number of adults; Mid-1981; (25) Qiryat Anavim (Jerusalem), Israel; 50–100 adults; September 1981; (26) Gorky, Soviet Union; small number of adults; Mid-1981; (27) Voronez, Soviet Union; small number of adults; Mid-1981; (28) Vitebsk, Soviet Union; small number of adults; Mid-1981; (29) Gavros-Achaia, Greece; >25♀♀; September 1981.

species. They list 21 previous studies, 19 of which cite data showing significant variation in mate recognition. However, they have a number of reservations regrading this result and it remains whether this is a general characteristic of natural populations or a result of experimental technique.

Our results can be interpreted in two possible ways. First, recent dispersion of *D. melanogaster* may have allowed little time for the development of genetic differences between populations. Alternatively, populations of *D. melanogaster* have indeed differentiated genetically in elements of their biology, but stabilising selection acting on the reproductive communication system has prevented it from diverging.

If there is an overall lack of divergence then our results are not surprising. However, recent research on the biology of natural populations of *D. melanogaster* has revealed considerable genetic differences, including biometry[16–20], electromorphs[21,22], chromosomal inversion frequencies[23,24], morphological mutant phenotypes[25], ecology[26,27], physiology[19]. These differences may be the result of selection in different local conditions. Recent investigations of hybrid dysgenesis[28–30] in *D. melanogaster* indicate that potential for 'postmating reproductive isolation' exists among geographically diverse populations. Why then have the genes controlling mating behaviour apparently resisted such change? Deviation in mating behaviour of laboratory populations of *D. melanogaster* has been recorded by Kilias *et al.*[31]. However, this may be due to non-genetic changes in mating rhythms which if corrected for, may result in no significant deviation for example ref. 32.

The second interpretation of our data is that stabilizing selection exerted through the interactions of the organisms themselves, acts on the reproductive communication system to maintain its stability. The function[33] of this male–female communication system is to facilitate the achievement of efficient syngamy in the conditions prevailing in the habitat of the organisms[34]. It has been argued (see ref. 33 for a recent view), that individuals with a deviant mate recognition system, would obtain less matings than those members of the population performing the correct sequences[34–36] that are necessary for successful reproduction. These deviant individuals would thus be less fit. It might be expected then that the mate recognition system should show a degree of geographic stability[37] and the data presented here support this prediction.

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Birth sex ratios and social rank in rhesus monkey mothers

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The ability to control the sex of her offspring could be of survival value to a mother[1,2], and prompts questions about mechanisms of sex determination. Trivers and Willard have suggested that mothers potentially able to invest much in offspring should bear those kinds of infant that repay high levels of maternal investment most effectively[2]. One hypothesis concerning matrilineal primate species states that a dominant mother would leave more descendants through a daughter rather than a son if that daughter inherited her high rank[3–6]. On the other hand, a low ranking mother of such a species would leave more descendants through a son, for he is likely to emigrate at puberty and not necessarily inherit his mother's low rank[6]. Unfortunately, long-term data on primate birth sex ratios are in short supply. Here we present data collected over 20 years on a captive colony of rhesus monkeys which support the above hypothesis. High ranking rhesus monkey mothers in our captive colony[7] were more likely to give birth to daughters than sons, while the remaining mothers were more likely to bear sons than daughters. Finally, we suggest a mechanism for adjusting birth sex ratios in macaques.

The conceptions and pregnancies referred to in this study occurred in six small social groups of rhesus monkeys (*Macaca mulatta*). Each group was housed in its own indoor room with adjoining outdoor run, at the Subdepartment of Animal Behaviour, near Cambridge[7]. There were 7–12 monkeys in each group—one adult male, two to five adult females (at least 4 yr old), and immature offspring born into that group. The groups shared the same building so that every monkey could see and hear every other one. As far as possible, families of mothers and their female offspring were kept in their original groups. We removed young males before their fifth years, and changed incumbent adult males often enough to prevent them from impregnating their daughters. In this paper our data exclude pregnancies resulting from matings arranged with males held apart from the mother's home group. Also excluded are pregnancies spent partly away from the home group.

Rhesus monkey mothers having high social ranks gave birth to twice as many daughters as sons (Table 1 (1960–82), $P = 0.0026$, binomial test[8]), while the remaining mothers bore more sons than daughters ($P = 0.024$, binomial test). (Among the non-high ranking mothers, middle and low ranking ones did not differ in their birth sex ratios.) The effect of maternal dominance on birth sex ratio was consistent from the 11-yr period preceding 1972 to a subsequent 10-yr period. Most mothers contributed more than one infant to Table 1, but when each mother was classified according to whether she bore more daughters than sons or vice versa, the contrast in birth ratios between high ranking and other mothers was still apparent (Table 2, $P = 0.03$, 0.03 and 0.001 for the three time periods, Fisher exact probability test). The effect also shows in first births to the mothers (Table 3, P = not significant, 0.02 and 0.10 for the three time periods, Fisher exact test), suggesting that it could be independent of a mother's previous reproductive history.

Table 1 Number of infants of each sex born to mothers of different ranks

Births between:	1960–71		1972–81		1960–81	
	Daughters	Sons	Daughters	Sons	Daughters	Sons
High rank mothers	18	6	20	9	38	15
Other mothers	7	15	25	39	32	54

A total of 53 mothers and 139 births are included in the sample. All conceptions and pregnancies occurred in the social group. Eight infants dying between their first and fourteenth days, and all available (16) sexed stillbirths are included. Social ranks of mothers living in small captive groups[7] were assessed before we knew of their possible connection with birth sex ratios. In practice, a high ranking mother is easily recognized as dominating the other females in her group. To represent the mother's rank at the likely time of conception, we used her rank when the resulting infant was 52 weeks old, for in only 20 cases did we have information about the mother-to-be's rank at conception. In none of these cases was there a change between the two rank positions. Information about the ranks of mothers with younger infants was not used because their greater dependence could have influenced their mothers' ranks. From 1960 to 1971, assessments of rank were made by earlier observers[22]. Thereafter, attacking, chasing, displacing, avoiding, fear grinning, and presenting (non-sexual, non-grooming context) between a pair of monkeys were regarded as evidence for dominance. In any dyad, one monkey was deemed dominant to the other if their actions occurred three or more times as often in one direction as in the other. A mother's rank reflects the direction but not necessarily the rate of aggressive behaviour between herself and others. A high ranking female would not be aggressive so long as the others avoided her, but the others could be fighting among themselves at a high rate.

Altmann[3] showed the same effect in free-living yellow baboons (*Papio cynocephalus*), her ratios being 18:10 daughters to sons for high ranking mothers, and 8:15 for the remainder. Silk's[4] low ranking (captive) bonnet macaques (*Macaca radiata*) gave birth to 20:44 females to males, although females from the top half of the hierarchy did not have more daughters than sons.

Until we know more about the mechanisms controlling birth sex ratios and the ways in which social factors operate[1], hypotheses about the adaptive significance of birth sex ratios

Table 2 For mothers holding only one rank across all their infants, the number of mothers of each rank who produced more daughters than sons, or sons than daughters

	1960–71		1972–81		1960–81*	
	More daughters	More sons	More daughters	More sons	More daughters	More sons
High rank mothers	7	2	5	0	9	0
Other mothers	3	10	5	10	8	17

Some mothers contributed infants to both time periods, and 13 mothers who changed rank were excluded.

* Six mothers (three high, three other) had equal numbers of daughters and sons.

must remain tentative, for the mechanism acting in our colony may be triggered by as yet unspecified special conditions in it. Meanwhile, we believe that our socially living mothers bias the birth sex ratios of their offspring according to some aspect of their dominance rank. Such a mechanism would have survival value for high ranking rhesus monkey mothers if daughters best repaid their mothers' high levels of maternal investment in them. This is a general form of the Trivers and Willard hypothesis[2] that mothers potentially able to invest much in offspring should bear and rear the kinds of infants repaying the highest levels of maternal investment most effectively. Such infants could be daughters in some socially living macaque and baboon species[3,4], although they were believed to be sons in the cases considered by Trivers and Willard.

In support of our view, rhesus monkey daughters remain with their mothers and inherit their mothers' social ranks[5], so that a high ranking daughter could contribute more to her mother's fitness by aiding in interfamily fights[9] and by breeding more successfully[10] than a son, who is likely to emigrate at puberty[11–13], and not necessarily achieve high social rank in the group he subsequently joins[6].

However, daughters bring costs as well as benefits to their mothers, for example by competing with each other within their families, and 1- and 2-yr-old females in our colony are especially likely to behave with jealousy and aggression to their mothers and subsequently born siblings[14]. Emigrating sons of high ranking mothers could take with them certain advantages gained from their mothers' statuses even if they did not retain their mothers' ranks.

Table 3 Number of infants of each sex born to primiparous mothers of different ranks

	1960–71		1972–81		1960–81	
	Daughters	Sons	Daughters	Sons	Daughters	Sons
High rank mothers	1	2	4	0	5	2
Other mothers	1	1	3	11	4	12

In the second time period, while 11 'other' mothers had sons as their first infant, only 10 went on to have more sons than daughters (see Table 2).

Functional arguments can be applied to low ranking mothers also. A daughter of a low ranking mother may share with her mother the disadvantages of low rank, including poorer breeding performance[15], while an emigrating son, whose rank is not thereafter tied to his mother's, may gain sufficient rank in his new social group to contribute considerably to his mother's fitness. In addition, young daughters may be more costly to rear than sons, being more involved in family strife, and bringing more aggression to their mothers from other adults[4]. Mothers were also slower to breed again after bearing daughters rather than sons[16].

However, these functional arguments depend partly on results from other social groups and species. To assess the balance of costs and benefits for high and low ranking mothers rearing sons and daughters, we need to know both the costs and the benefits to individuals through the reproductive lives of themselves and their offspring[10].

The mechanisms whereby these sex ratios are controlled is unknown[1]. Differential effects of stress on the proportions of XX- and XY-bearing sperm reaching the ovum[3], or on the post-conception survival of male and female fetuses[17] would explain our bias away from daughters in the births to low ranking mothers, if XX-sperm and/or female fetuses were more vulnerable, and low rank were associated with greater stress. Potentially stressful situations, however, did not increase the chance that our mothers would bear sons: 10 mothers scoring above the median on both aggression received from other adults and submissive behaviour given to others (see Table 1) did not give birth to more sons than daughters. Moreover, a stress hypothesis could not explain our bias towards female births in high ranking mothers.

We suggest that rhesus monkey mothers, like white-tailed deer[18], could adjust the sex ratios of their infants if they controlled the timing of mating relative to ovulation. At present we know of no supporting evidence from non-human primates, although in human beings weak biases towards males have occurred when zygotes are formed early or late in the fertile period rather than in the middle[19], and biases towards females occurred when gonadotropin levels were deliberately made high[20]. The question of how the timing of female sexual behaviour close to the time of ovulation depends on the mother's social situation[21] remains open.

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Use of the UCHT1 monoclonal antibody to explore mouse mutants and development

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Garson and Coakham demonstrated a monoclonal antibody (UCHT1) which specifically labels Purkinje cells in the cerebellar cortex[1]. To investigate mouse mutants with cerebellar lesions, we obtained some of the purified antibody from Beverley[2], who derived it as an antibody to a human T-cell lymphocyte surface antigen. We have used the indirect immunoperoxidase method to label with the antibody Purkinje cells in mutant and normal mice, frog, toad, goldfish, dogfish and 3-acetylpyridine-treated rat. We report here that in the mutant mice tested, only the Purkinje cells in *staggerer* did not stain. The Purkinje cells of frog, toad and goldfish were also unstained but those in the dogfish were labelled. The Purkinje cells in rats which had been injected with 3-acetylpyridine to destroy the climbing fibres, were found to stain with the antibody. These results suggest phylogenetic as well as ontogenetic differences in antigenicity of Purkinje cells.

The cerebella in the mutant mice used all have different, well defined lesions: *stumbler* Purkinje cells have stunted dendritic trees[3]; *Lurcher* loses all its Purkinje cells in the first 60 days abter birth[4]; *weaver* loses all of its granule cells and has abnormal Purkinje cell dendritic trees[5]; *reeler* has a disorganized

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cerebellar cortex with Purkinje cells in abnormal positions[6], and *staggerer* has reduced numbers of Purkinje cells[7].

Mutant and littermate control animals were all aged between 15 and 20 days postnatal when used for these initial experiments because *Lurcher* begins to lose significant numbers of Purkinje cells after 15 days. The *stumbler, Lurcher, weaver* and *reeler* mutants all showed positive staining of Purkinje cells with UCHT1. In all cases the whole of the Purkinje cell was labelled; the cell body (excluding the nucleus), the dendritic tree, and the axon, which could be traced in some cells all the way to the deep cerebellar nuclei. With this labelling, the abnormal Purkinje cell dendritic trees were seen as described previously in Golgi preparations[3,4,8,9].

In the *staggerer* mutant, however, the Purkinje cells were completely unstained. Figure 1*a, b* shows sections of a control and *staggerer* cerebellum stained with UCHT1 using the indirect immunoperoxidase technique. These sections were not counterstained and were photographed using Nomarski optics to allow Purkinje cells unlabelled by antibody to be seen in the *staggerer* section (Fig. 1*b*).

There was a difference in staining between the control and *stumbler* Purkinje cells. In control animals the staining was uniform whereas in *stumbler* the horseradish peroxidase (HRP) gave a clumped appearance with numerous small clear areas (Fig. 1*c, d*). These abundant clear areas are almost certainly due to the large numbers of mitochondria present in the soma of *stumbler* Purkinje cells[3].

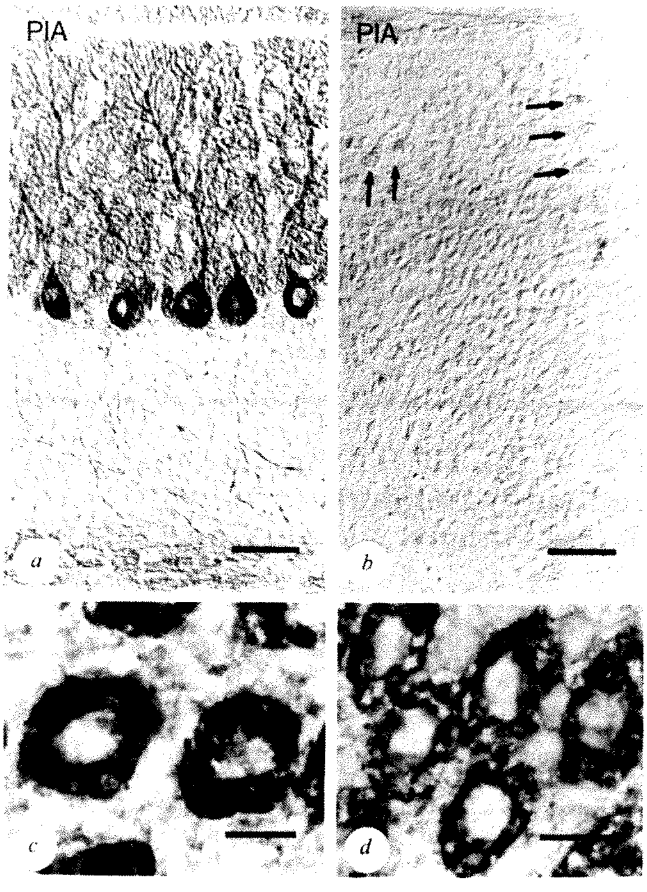

Fig. 1 *a, b,* Sagittal sections of cerebellum of 20-day-old normal and *staggerer* mice, respectively, stained by UCHT1 using the indirect immunoperoxidase technique. Cryostat sections (6 μm) were fixed for 5 min in acetone, incubated for 30 min with a purified IgG fraction of the UCHT1 ascites diluted 1:1,000, washed for 5 min in Tris-buffered saline, and then incubated for 30 min at 20 °C with HRP-conjugated rabbit anti-mouse immunoglobulin (DAKO; 1:100). The peroxidase was visualized using 0.6 mg ml^{-1} diaminobenzidine. Sections were photographed with a Leitz orthoplan microscope and Vario-orthomat camera using Nomarski optics. Arrows in *b* indicate unstained Purkinje cells. Scale, 50 μm. *c, d,* Sagittal sections of Purkinje cells from 19-day-old normal and *stumbler* mice, respectively, stained with UCHT1 and counterstained with haematoxylin. Note that the HRP appears more clumped with numerous small clear areas in the *stumbler* Purkinje cells because of the increased numbers of mitochondrial profiles in these cells. Scale bar, 10 μm.

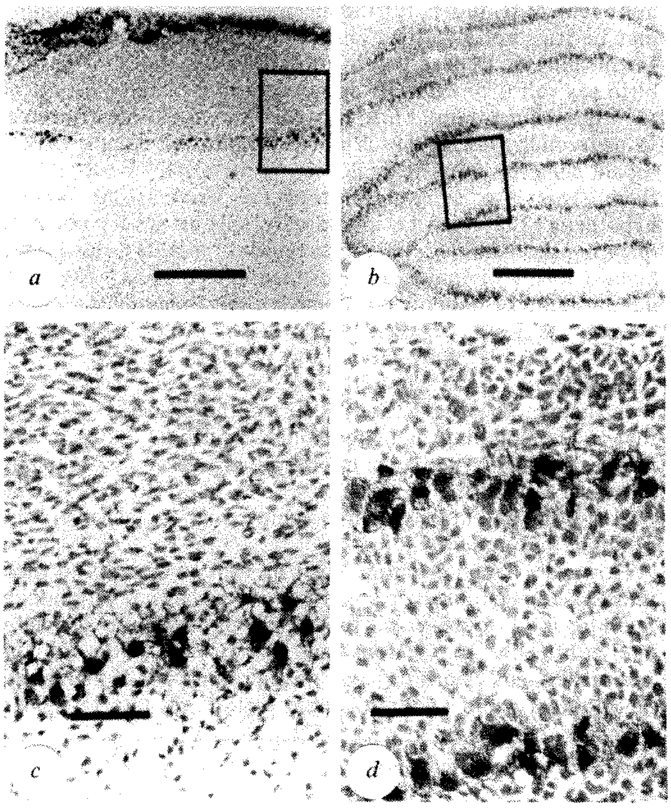

Fig. 2 *a, b,* Horizontal 6-μm sections of 2- and 3-day-old normal mice cerebella stained with UCHT1 and counterstained with haematoxylin. Note the patchy staining of Purkinje cells in *a*. Low power bright field; scale bar, 500 μm. *c, d,* Areas within rectangles in *a* and *b* shown at higher magnification. Scale bar, 50 μm.

We also studied the labelling pattern of normal mice aged 0 (birth), and at days 1, 2, 3, 5 and 8 postnatal. At time 0 and on day 1 the Purkinje cells were found not to stain with UCHT1 but at day 2, patches of stained Purkinje cells were clearly visible (Fig. 2*a, c*). At day 3 (Fig. 2*b, d*) and for all subsequent ages, the Purkinje cells stained as in the day 20 normal control, except that the Purkinje cells at less than 8 days old were noticeably less mature.

We know that between 2 and 3 days postnatal, the age at which the particular antigen appears, the mouse climbing fibres first start to form synapses on the Purkinje cells via somatic spines (K.W.T.C., unpublished observations). Two studies[10,11] indicate that although the synapses of the climbing fibres are present in the *staggerer*, they are not functional except after injection of harmaline; this suggests that the presence of climbing fibres may be correlated with the presence of the antigen. To examine this hypothesis, we treated rats with 3-acetylpyridine, harmaline and nicotinamide[12] to destroy selectively the inferior olivary nuclei, which are believed to be the sole source of climbing fibres[13]. Our experiments showed no change in the Purkinje cell labelling in any part of the cerebellar cortex. We conclude that the continued presence of climbing fibres is not a requirement for the presence of the antigen in Purkinje cells.

The possibility remains that the appearance of the antigen correlates with the appearance of the climbing fibre innervation. This would explain our findings with the young animals but not the results with the *staggerer* mutant. In *staggerer* it has been shown that the development of the Purkinje cells is retarded[14] to the extent that climbing fibre synapses are still present on somatic spines of animals 23 days after birth. It has also been shown that *staggerer* Purkinje cells have no parallel fibre–dendritic spine synapses[15]. This failure to form branchlet spines in *staggerer* is almost certainly due to some inherent defect in the Purkinje cells since these cells have been shown to be the site of action of the mutant gene[16]. Whether or not the absence of the antigen in *staggerer* is causally related to the *staggerer* disorder is an open question.

To determine any further possible correlation between the presence of the antigen and the structure of the cerebellum, we have extended the survey of the animal kingdom begun by Garson *et al.*[1]. The species tested were frog (*Rana temporaria*), toad (*Rana esculenta*), goldfish (*Carassius auratus*), dogfish (*Scyliorhinus canicula*) and pigeon. We used the indirect immunoperoxidase method to label Purkinje cells in at least two of each of these species. Although this study is at best preliminary, it is of interest to note that only in the dogfish and pigeon did the Purkinje cells stain. This finding is interesting because Llinas and Hillman[17] have shown that the cerebellum of the elasmobranchs is more complex than that in frog and other amphibia. By this they mean that there are fewer cell types in the frog (basket cells missing) and the Purkinje cells are less complex. In fact, the Purkinje cells in the frog have far fewer spiny branchlets on their dendrites than elsasmobranchs and probably present a more primitive form of cerebellar cortex[17]. The similarity in the gross morphology between *staggerer* and frog Purkinje cells may be of importance in this context.

It seems that the antigenic determinant for UCHT1 is absent from some species, from at least one mutant mouse and also from mice less than 2 days old. At this stage it is impossible to correlate the antigen with any structure in Purkinje cells but we hope that further electron microscope and biochemical studies may elucidate its location.

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Meitoic crossing-over between the X and Y chromosomes of male mice carrying the sex-reversing (*Sxr*) factor

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In the mouse, as in many other species, the X and Y chromosomes are seen to pair end-to-end at diakinesis[1], which has led to speculation as to whether chiasmata and crossing-over occur earlier in meiosis[1]. Arguments in favour of this have recently been presented[2–4], but because of the absence of appropriate genetic and cytological markers little clear-cut evidence has been obtained. Studies with 'sex-reversed' (*Sxr*) mice[5] carried out earlier this year[6] have suggested that the transfer of chromatin from the Y to the X chromosome takes place during meiosis, but in this instance the interpretation was confused by the claim that X/Y *Sxr* mice possess two Y chromosomes, contrary to earlier reports[7]. We present evidence here that: (1) X/Y *Sxr* mice have a single abnormal Y chromosome possessing an additional dark-staining distal body; (2) during meiosis, this body is transferred by crossing-over from one chromatid of the Y to one chromatid of the X. Furthermore, closer inspection of XY bivalents in mice not carrying *Sxr* suggests that crossing-over is a normal feature of X–Y pairing, and may be a necessary requirement for the production of functional sperm. Details of the mode of inheritance of *Sxr* are given in Table 1.

Thorough examination of the karyotype of X/Y *Sxr* males and their X/Y non-*Sxr* sibs showed no gross departure from the standard XY karyotype, and it was concluded that the claim for the existence of an X, Y, Y1 system[6] resulted from misinterpretations of the precocious separation of the Y chromatids at mitotic metaphase when prolonged colcemid treatments are employed (see also refs 2–4), and of the unusual configurations adopted by the X and Y chromosomes in the sex vesicle during meiotic prophase[8,9].

Detailed analysis of the mouse Y chromosome is difficult as in metaphase of mitosis it frequently shows uniformly dense staining after G-banding[10], and even if bands are revealed they are often distorted by coiling. In the accepted nomenclature[10] the chromosome is described as being composed of five major bands (Fig. 1*a*) but the authors included the proviso that examples from some strains might reveal variant banding patterns. We would add that many, if not all, mouse Y chromosomes have a short arm[11], and in the case of the elongated examples from the control mice studied here, reveal further bands on their long arms (Fig. 1*b*).

Examination of the Y chromosome of X/Y males from the *Sxr* stock (Table 1) revealed that whereas X/Y non-*Sxr* animals possessed a normal appearing Y, the Y of X/Y *Sxr* males possessed an additional constricted dark-staining distal band or body (Fig. 1*c*). In midmetaphase this caused the proximal and distal ends of the Y to be so similarly marked that it became difficult to orientate the chromosome with respect to its centromere. However, the distal body stained in a reverse manner to the centromeric or proximal region of the chromosome, being dark in the early stages and becoming progressively lighter as metaphase proceeds.

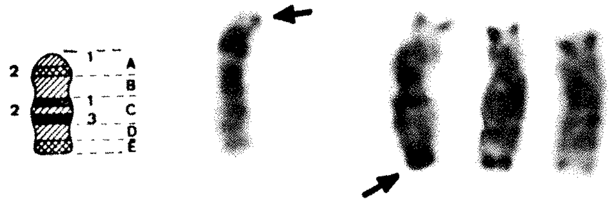

Fig. 1 *a*, The accepted standard idiogram of the mouse Y chromosome[12]. *b*, G-banded, elongated Y from a bone marrow cell of a normal (control) XY male mouse, showing evidence of a short arm (arrowed) and further bands on the long arm. Control mice are derived from the 101/H, Ju/FaCt and Q (Falconer) strains among which no clear Y chromosomal differences were found. *c*, G-banded Y chromosomes from bone marrow cells of an X/Y *Sxr* mouse. Left: in early metaphase, showing the additional, heavily stained, distal body (arrowed). Centre: in midmetaphase, showing the equivalent staining of proximal and distal ends. Right: in late metaphase, showing the fainter staining of the distal body with mitotic progression.

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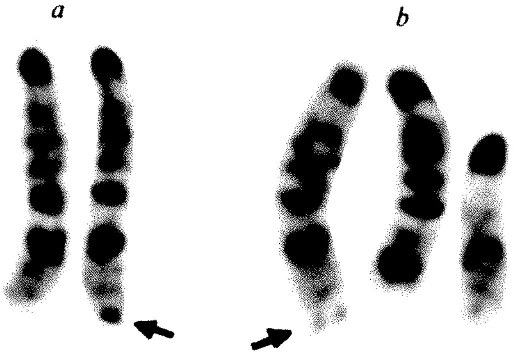

Fig. 2 *a*, G-banded X chromosomes from a bone marrow cell of an X/X *Sxr* male. Left: the normal X; right: the X has an additional, dark-staining, distal body (arrowed). *b*, G-banded X chromosome from a bone marrow cell of an X/X *Sxr* male carrying the T(X: 16)16H X-autosome translocation. Left: the paternally-derived normal X showing the distal body (arrowed).

The X chromosome in mitotic cells of X/Y *Sxr* males was observed to conform to the normal banding pattern of this chromosome[10], as were both X chromosomes from their X/X daughters. However, although one X of their X/X *Sxr* sons also looked normal, the other X possessed a dark-staining body at its distal end (Fig. 2*a*). Furthermore, in X/X *Sxr* males carrying the T(X;16)16H X-autosome translocation it was always the paternally-derived, non-translocated X chromosome that carried this body (Fig. 2*b*).

Meiotic studies at diakinesis in X/Y non-*Sxr* males (the X/Y progeny of X/Y *Sxr* mice shown in Table 1) and X/Y (from unrelated stocks) controls showed that in most spermatocytes the X and Y chromatids paired distally with a clear line of separation between them (Fig. 3*a*). In X/Y *Sxr* males the pairing was also distal but one pair of the X and Y chromatids showed more contact by way of two dark staining bodies (Fig. 3*b*), one on the X and the other on the Y. The bodies were always adjoining and not diagonally opposed, which indicated that a chiasmate exchange had occurred whereby the body had crossed over from one chromatid of the Y to one of the X. Confirmatory evidence of the exchange was provided by metaphase II observations of X and Y chromosomes with single chromatids marked by the dark-staining distal body. Another feature of the first meiotic division of X/Y *Sxr* males is a higher than normal frequency of X–Y univalence which, at diakinesis, may vary from 70 to 90%, as against 5 to 10% in the X/Y non-*Sxr* sibs[7]. In many, if not all, spermatocytes with X–Y univalence dark-staining bodies were observed on both chromatids of the Y with none of the X (Fig. 3*c*), suggesting that pairing failure in meiotic prophase gives no opportunity for transfer.

Our observations with *Sxr* mice indicate that *Sxr* represents a Y rearrangement in which part or all of the male determining region has been transferred from a relatively proximal location on the chromosome to the distal end where, by crossing-over, it can be transferred to the X and thus cause male development in X/X *Sxr* mice (see also refs 2–4). Our mitotic observations with X/Y *Sxr* animals, however, showed no evidence of any chromosomal change in the Y other than the addition of the distal, *Sxr* body. We therefore conclude that the original rearrangement involved either, (1) part of a multiple copy sex-determining region the loss of which from its normal location we cannot distinguish cytologically, or, (2) all or most of this region transferred by excision from one chromatid[5] and attached onto the distal end of the other. Our meiotic observations clearly show that *Sxr* is inherited in a simple manner after the formation of a regular chiasma and a cross-over taking place between the distal regions of the X and Y. Such a configuration produces four different classes of chromatid, X, X with *Sxr*, Y and Y with *Sxr*, which, when transmitted by the gametes, satisfies the segregation ratios observed in the progeny of X/Y *Sxr* males[7]. Pairing failure gives no opportunity for crossing-over and is manifested by X–Y univalence which, from our metaphase II observations appears to act as a cell lethal.

It may seem surprising in view of the many past cytological studies on *Sxr* mice that the *Sxr* body and its Y to X transfer has gone undetected. In part this may have been due to the fact that the apparent autosomal dominant inheritance focused attention primarily upon the autosomes. However, at the mitotic level, failure may also have been due to the staining vagaries of both the Y and the distal, *Sxr* body; at the meiotic level, to the best of our knowledge, no G-band analyses have previously been attempted on the condensed stages and at the synaptonemal complex (SC) level, any possible differences between X and Y pairing in carrier and non-carrier mice might have been obscured by synaptic adjustment[12].

The question of greatest significance which remains is whether crossing-over between the X and Y chromosomes is limited to X/Y *Sxr* males, or is typical of all mouse XY bivalents. Reappraisal of normal XY bivalents in the light of our *Sxr* findings suggests that with some there is evidence of an asymmetry caused by a greater continuity of adjoining pairs of X and Y chromatids (Fig. 3*d*), and this could be taken as evidence of a residual chiasma.

However, this behaviour was only observed in a minority of cells. The apparent inconsistency may be resolved by examining the available evidence for the cytologically visible amount of chromatin expected to be exchanged by crossing-over. Studies at the SC level[13] suggests that there is considerable pairing segment between the X and Y chromosomes and the possibility of a fairly large exchange. However, this can be excluded for the following reasons: (1) a cytological reassessment of the large paracentric inversion of the X chromosome[14], In(X)1H, identifies the distal break-point as being in band F3, or even F4, which is the terminal band, and despite this, the sex-bivalent

Table 1 The inheritance of *Sxr*

Cross	X/X♀	×	X/Y *Sxr* ♂
Progeny	X/X♀		X/Y♂
	X/X *Sxr* ♂		X/Y Sxr ♂

The sex-reversed (*Sxr*) factor causes chromosomal females to develop as phenotypically normal, but sterile males[5]. Transmission is through X/Y carrier males and, hence, all X/Y males, whether carrying *Sxr* or not, normally have the same Y chromosome. The mode of inheritance appears to be that of an autosomal dominant, but all attempts to locate *Sxr* on an autosome have failed[7]. A location in an homologous region of the X and Y chromosomes could give the same pattern of inheritance, however[2]. The observed segregation ratios accord well with expectation, the X/X and X/Y classes of progeny usually being distinguished with the aid of an X chromosomal marker gene. Stock maintenance is by crossing X/Y *Sxr* males × F_1 hybrid (C3H/HeH♀ × 101/H♂) females.

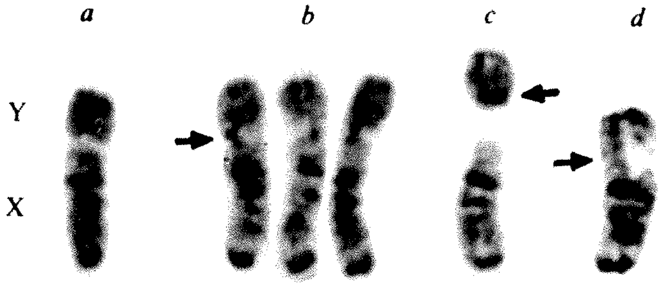

Fig. 3 G-banded X and Y chromosomes at diakinesis. *a*, From a control X/Y male showing the distal pairing of X and Y with a clear line of separation. *b*, Three examples from an X/Y *Sxr* male showing a greater continuity of one pair of adjoining X and Y chromatids by way of two dark staining bodies (arrowed). *c*, Univalent X and Y from an X/Y *Sxr* male showing the retention of the dark staining bodies on both chromatids of the Y (arrowed). *d*, Sex-bivalent from an X/Y non-*Sxr* male showing the greater continuity of an adjoining pair of X and Y chromatids (arrowed) suggesting earlier chiasma formation.

in male carriers shows a near-normal frequency of X–Y pairing at diakinesis, (2) cream (*Crm*), the most distal gene on the mouse X chromosome, lies outside this inversion[16] and yet in male mice with or without the inversion it has never been found to cross-over onto the Y (Lyon, M. F., personal communication). The amount of chromatin visibly available for reciprocal exchange is therefore minimal and the chiasma between the X and Y must be near-terminal, as has previously been suggested from SC studies[16]. It is therefore not surprising that the chiasma is displayed at diakinesis as 'terminalized' and the bivalent as symmetrical in the majority of primary spermatocytes of normal males which do not have the *Sxr* body to serve as a visible cross-over marker. We conclude that in all male mice a small reciprocal exchange between the X and Y is a regular and necessary requirement of meiosis for the production of functional spermatozoa.

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Male, female and intersex development in mice of identical chromosome constitution

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In the preceding paper[1], cytological evidence is presented to show that in 'sex-reversed' XX male mice[2] the sex-reversing factor, *Sxr*, is carried on a dark-staining body located on the distal end of the paternal X chromosome. The X-chromosomal location of *Sxr* raises the possibility that it may be subject to the X chromosome inactivation process[3]. However, the regular segregation of *Sxr* and the absence of any indication that intersexes occur with a higher than normal frequency in *Sxr* stocks[4] suggests that either inactivation of *Sxr* does not occur, or, if it does, that the proportion of cells with the *Sxr*-bearing X genetically active is sufficient to ensure normal testicular differentiation. Here we present information on the sexual development of chromosomally XX mice carrying *Sxr* in which the *Sxr*-bearing X chromosome is held in the inactive condition in most if not all somatic cells by use of the T(X; 16)16H (T16H) X-autosome translocated chromosome[5–10] Mice of this genotype were found to develop as normal but sterile males, normal fertile females or intersexes.

That T16H/X *Sxr* animals can develop as males was indicated by two observations. First, the sex ratio among the T16H progeny of the cross of T16H/X females with X/Y *Sxr* males (36:87, Table 1) was not significantly different ($\chi^2_1 = 0.98$) from the 1:3 (females:males) ratio normally found in *Sxr* stocks[4], and also evident among the non-T16H progeny of the cross 36:91, Table 1). Second, examination of the testes of 61 of the 87 males carrying T16H revealed that 17 lacked germ cells and were thus genetically T16H/X *Sxr*[2], and that the remaining 44 possessed germ cells, and were therefore genetically either T16H/Y or T16H/Y *Sxr* (Table 1). Cytological confirmation of the chromosome constitution was obtained in several animals. The T16H/X:T16H/Y plus T16H/Y *Sxr* ratio (17:44) agrees well with the 1:2 expectation ($\chi^2_1 = 0.59$), and provides little reason to suspect that T16H/X *Sxr* animals might develop other than as males. The data at this point therefore suggest that *Sxr* is not inactivated when the X on which it is located is inactivated.

Table 1 Breeding of T16H/X *Sxr* mice

Parental genotypes	T16H++/++*Blo* ♀ × +*Ta*+/Y *Sxr* ♂	
T16H progeny	Expected sex	No. observed*
T16H++/+*Ta*+	♀	36 ♀
T16H++/+*Ta*+ *Sxr*	♀ or ♂?	
T16H++/Y	♂	87 ♂
T16H++/Y *Sxr*	♂	
Non-T16H progeny		
++*Blo*/+*Ta*+	♀	36 ♀
++*Blo*/+*Ta*+ *Sxr*	♂	34 ♂
++*Blo*/Y	♂	57 ♂
++*Blo*/Y *Sxr*	♂	

* Based on external sexual phenotype.

Investigation of the T16H females derived from the cross provided reason to modify this conclusion, however. On being test-mated with a normal male, one female produced, among a total of 21 progeny, five X/X *Sxr* sons and two T16H/X *Sxr* sons (Table 2). Clearly, this female must have carried *Sxr*, and this was confirmed by the cytological observation of the *Sxr* body on its normal X chromosome. The occurrence of the T16H/X *Sxr* sons, and also of two non-T16H daughters (Table 2), incidentally provided evidence of meiotic recombination between the T16H breakpoint and *Sxr* in the mother, transferring *Sxr* from the normal X to the T16H X chromosome. Eight other fertile females have now been examined cytologically, and two carried *Sxr*. Thus, among a total of 20 T16H/X *Sxr* animals so far examined, 3 have developed as females and 17 as males. The female development of some T16H/X *Sxr* animals indicates that *Sxr* must be subject to the X-inactivation process in some cells.

Further evidence that *Sxr* can be subject to X-inactivation was obtained from more detailed examination of the T16H/X *Sxr* males. Although all 17 appeared unambiguously male, internally as well as externally, four possessed testes which displayed abnormalities greater than those typically found in X/X *Sxr* (that is, non-T16H) males: their testes tended to be smaller, their tubules appeared more difficult to tease apart than the equivalent sterile tubules of X/X *Sxr* males, and, most strikingly, they were partially covered by blood clots which extended to the epididymis and proximal regions of the vas deferens. One testis from each of these males has been examined histologically and in each one a minor (5–10%) ovarian component was identified. The remaining 13 males possessed histologically normal testes. The occurrence of intersex T16H/X *Sxr* mice is consistent with McLaren and Monk's[11] discovery of three hermaphrodites, with a testis on one side and an ovary on the other, among 10 T16H/X *Sxr* mice.

Two of the three T16H/X *Sxr* females identified have also been examined in detail. In both, the ovaries and reproductive organs were macroscopically normal and the structure of the ovaries was histologically normal. Clearly, therefore, T16H/X *Sxr* mice may show a range of phenotypes from normal male, through intersex, to normal female. An incidental point is that the fertility of the T16H/X *Sxr* females shows that the presence

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Table 2 Progeny of a fertile T16H/X *Sxr* female

Cross: T16H++/+*Ta*+ *Sxr* ♀ × ++*Blo*/Y ♂

XX progeny		XY progeny		
Genotype	No.	Genotype	No.	
T16H++/++*Blo* ♀	1	T16H++/Y ♂	?	9
T16H++ *Sxr*/++*Blo* ♂*	2	T16H++ *Sxr*/Y ♂*	?	
+*Ta*+ *Sxr*/++*Blo* ♂	5	+*Ta*+ *Sxr*/Y ♂†	1	2
+*Ta*+/++*Blo* ♀*	2	+*Ta*+/Y ♂*	?	

* T16H–*Sxr* recombinant classes. Recombination frequency 36.5% (4/11).

† Novel XY genotype in which *Sxr* must be carried on the X rather than on the Y.

in the oocytes of the male-determining (*Sxr*) DNA sequences in a form which is presumably genetically active (as both X chromosomes are reactivated at the onset of meiosis[12,13]) does not interfere with their ability to form functional oocytes. This observation is consistent with the finding[14] that XY germ cells could form functional oocytes in a female XX↔XY mouse chimaera.

The gene markers used in the T16H cross (Table 1) clearly showed that the presence of *Sxr* did not modify the non-random X-chromosome activity in the T16H heterozygote. The range of sexual development in animals of a single (T16H/X *Sxr*) genotype therefore suggests that the primary cause is a variable inactivation of *Sxr* in the inactivated *Sxr*-bearing X, generating *Sxr*-active and *Sxr*-inactive populations of cells. As such this may represent a further example of position effect variegation in mammals[15], so far only observed with autosomal loci in certain X-autosome translocations[16,17]. Clearly, secondary factors also must operate in T16H/X *Sxr* mice to influence the final sexual phenotype, and these would include frequency and distribution of the two cell types, the ability of the *Sxr*-active cells to induce *Sxr*-inactive cells to form testicular tissue, perhaps through H–Y antigen coating (as postulated to occur in XX↔XY chimaeras[18]) and also the number of cells in the fetus that form the primordial gonad.

The fact that male development occurs at all in T16H/X *Sxr* mice where the *Sxr*-bearing X is inactive in virtually all somatic cells, indicates that *Sxr* must remain active, although on an inactive X, in a high proportion of cells. Thus, with random X-inactivation, the proportion of cells with *Sxr* inactive must be very low, and it is therefore likely that X/X *Sxr* animals seldom, if ever, develop as females or intersexes due to the presence of *Sxr*. The possibility is, however, being investigated in X/X *Sxr* animals which, because of X chromosome controlling element (*Xce*) allele heterozygosity, have the *Sxr*-bearing X active in only about 30% of their somatic cells[19].

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Fertile females produced by inactivation of an X chromosome of *'sex-reversed'* mice

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Sex-reversed (*Sxr*) is a dominant mutation that confers maleness on X/X *Sxr* mice[1]. Although its mode of inheritance is typically autosomal, all attempts to map *Sxr* to an autosomal chromosome have failed[2]. P. Burgoyne (personal communication) suggested that if *Sxr* were located at the end of the sex chromosomes, distal to a postulated obligatory cross-over in the X–Y pairing segment, it would show such an apparent autosomal pattern of inheritance. To test whether *Sxr* is located on one of the X chromosomes, and is perhaps therefore subject to X-chromosome inactivation, we have examined mice heterozygous for an X-autosome translocation, T(X; 16)H (ref. 3), such that the normal X chromosome postulated to carry *Sxr* is preferentially inactive. Independently, Singh and Jones[4] have now shown by *in situ* hybridization that *Sxr* consists of a duplicated section of Y chromosome material that is indeed translocated to the distal terminus of one X chromatid during male meiosis[5,6]. Our results show that when the X chromosome carrying *Sxr* is preferentially inactivated, fertile T(X; 16)H/X *Sxr* females can be produced, owing to an associated inactivation of the male-determining *Sxr* sequences.

Females carrying T(X; 16)H, hereafter referred to as T16H, and heterozygous for the X-linked enzyme marker *Pgk-1*[7,8], were mated to males carrying *Sxr* and an X-chromosome marker (Table 1). The genotypes of the progeny were deduced as indicated in Table 1. PGK-1 phenotype was determined in order to classify the maternally inherited X chromosome (T16H *Pgk-1*^{b} or X *Pgk-1*^{a}). Male and female *Sxr*-carrying progeny were identified by the presence in their progeny of males with variegated coats after test mating with partners carrying an X-linked coat pattern marker. Sterile males were analysed by a combination of testis weight, testis histology and sex chromosome constitution, to distinguish between T16H/Y males in which spermatogenesis is arrested, and X/X *Sxr* males in which it is absent. The results are shown in Table 2. Progeny inheriting the normal X chromosome from the mother served as controls for those inheriting the T16H chromosome. A deficiency of *Sxr* carriers was observed among the progeny regardless of which X chromosome they inherited from the mother. All the female progeny were fertile. All the X/X *Sxr* progeny (13/13) were sterile males, showing typical X/X *Sxr* testicular histology[1]. However, of the T16H/X *Sxr* progeny, five were sterile males, two developed as fertile females, and three proved to be hermaphrodites, with a testis on one side and an ovotestis on the other. The ovotestes were very small, and contained oocytes, sometimes in well developed follicles, within abnormal testicular tubules.

Identification of the two females carrying *Sxr* was obtained by progeny-testing. Table 3 shows the data for one of the females, which was mated first to a Gs/Y and then to a Ta/Y male. The female produced two phenotypically striped X/X *Sxr* sons, and hence was carrying *Sxr*. The overall sex ratio (23♂:11♀) differs significantly from 1:1 ($P<0.05$), but not from the 3:1 ratio expected from an *Sxr*-carrying female. Moreover, amongst the non-variegated male young five T16H *Sxr*/X males (non-variegated owing to non-random X-chromosome expression) were identified, indicating that *Sxr*, initially present on the *Pgk-1*^{a} X chromosome, may be transferred to the T16H chromosome during oogenesis. The proportion of XX progeny inheriting *Sxr* from the mother (7/18) is consistent with the 50% expected from an *Sxr* heterozygote.

The finding that *Sxr* sometimes fails to be expressed has been confirmed by Cattanach and his colleagues[9], who have found

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Table 1 Characters scored in the genotype analysis of the progeny of the parental cross T16H *Pgk-1*^{b}/+*Pgk-1*^{a} ♀ × *Pgk-1*^{a}/Y*Sxr* ♂

Phenotypic sex	PGK-1 type*	Fertility†	Analysis of sterile males‡: Testis weight (mg)	Analysis of sterile males‡: Spermatogenesis (histology)	Analysis of sterile males‡: Karyotype	Appearance of *Sxr* in F_2 progeny§: Variegated XX *Sxr* males	Appearance of *Sxr* in F_2 progeny§: Non-variegated T16H/X *Sxr* males	Genotype
♀	A	Fertile	—	—	—	Absent	—	+*Pgk-1*^{a}/+*Pgk-1*^{a}
♀	B	Fertile	—	—	—	Absent	Absent	T16H *Pgk-1*^{b}/+*Pgk-1*^{a}
♀	B	Fertile	—	—	—	Present	Present	T16H *Pgk-1*^{b}/+*Pgk-1*^{a} *Sxr*
♂	B	Sterile	<25	Absent	XX	—	—	
♂	B	Sterile	10–50	Arrested	XY	—	—	T16H *Pgk-1*^{b}/Y, or Y *Sxr*
♂	A	Fertile	—	—	—	Absent	—	+*Pgk-1*^{a}/Y
♂	A	Fertile	—	—	—	Present	—	+*Pgk-1*^{a}/Y *Sxr*
♂	A	Sterile	<25	Absent	XX	—	—	+*Pgk-1*^{a}/+*Pgk-1*^{a} *Sxr*

The female mice in the parental cross were derived from crosses between a stock carrying T16H, obtained originally from MRC Radiobiology Unit, Harwell, and a stock carrying *Pgk-1*^{a}, a variant allele at the X-linked locus coding for the monomeric enzyme PGK-1 (phosphoglycerate kinase 1; EC2.7.2.3), obtained originally from John West, Zoology Department, Oxford. The male mice in the parental cross carried either *Pgk-1*^{a} or one of the X-linked coat-pattern markers *Ta* (Tabby), *Hq* (Harlequin) and *Gs* (Greasy), obtained originally from the Institute of Animal Genetics, Edinburgh. The table outlines the strategy used for classifying genotypically the progeny derived from *Pgk-1*^{a}/Y *Sxr* males; a similar strategy was used for the progeny of *Ta*/Y, *Gs*/Y and *Hq*/Y males. The strategy also allowed the identification of recombinant progeny; these have not been included in the table.

* PGK-1 (A, B or AB) was determined by gel electrophoresis[28] of blood samples taken from the retro-orbital sinus.

† To test fertility, each of the male progeny was mated to two females carrying *Ta*, *Gs* or *Hq*, and each of the female progeny was mated to a male carrying one of the same three markers. Inspection of the coat pattern of the young of fertile animals allowed *Sxr* carriers to be identified (see §) and XX to be distinguished from T16H/X females (the latter have some non-variegated daughters).

‡ Sterile males were killed and their testes weighed. A plot of mean testis weight against body weight allowed X/Y to be distinguished reliably from T16H/Y males. Non X/Y testes weighing at least 25 mg were classified as T16H/Y; below 25 mg there was a region of overlap between the smaller T16H/Y and the larger X/X *Sxr* testes. These testes were therefore analysed histologically (spermatogenesis is absent in X/X *Sxr* and arrested in T16H/Y males); in most cases the mice were also karyotyped from bone marrow preparations. We could not distinguish T16H/Y *Sxr* from T16H/Y sterile males.

§ Fertile males and females carrying *Sxr* were identified by the presence of variegated (striped) males (X/X *Sxr*) among their progeny (for matings, see †). In the case of PGK-1B females (putatively T16H), non-variegated male progeny were also screened by the analysis described above ‡, since the T16H/X *Sxr* sons, although carrying a *Ta*, *Gs* or *Hq* gene on their normal chromosome, would not be variegated.

another fertile T16H/X *Sxr* female, shown to be an *Sxr* carrier by progeny testing, as well as two other T16H/X *Sxr* females identified cytologically. The occurrence of such females among T16H/X *Sxr* but not X/X *Sxr* individuals (Table 2) suggests strongly that the failure of expression is due to inactivation of the *Sxr* male-determining region as a consequence of its attachment to the normal X chromosome, preferentially inactive in the presence of T16H.

The observation that some T16H/X *Sxr* individuals develop as females, some as males, and some as hermaphrodites, is most simply explained by assuming that the *Sxr* male-determining region is inactivated in some cells but not others. Inactivation may spread to a variable extent from the inactive X chromatin into the attached *Sxr* fragment, in an analogous fashion to position effect variegation for autosomal genes translocated onto the inactive X chromosome[10]. We assume that the extent of spreading leading to active or inactive *Sxr* status is a heritable characteristic determined at the time of X inactivation. The sex of each individual will then depend on the proportion of cells expressing *Sxr* in the gonad primordium.

Studies on aggregation chimaeras suggest that a certain critical proportion of XY male-determining cells is required for a mixed gonad to develop as male. Of 31 adult XX↔XY chimaeras reported in 5 different strain combinations[11–15], 21 were male, 7 female and 3 hermaphrodite. Since the relative proportions of the two cell components appear to show a flat distribution from 0 to 100% in aggregation chimaeras[16], this would indicate (ignoring variation from one strain combination to another) that the critical threshold above which development of a normal male gonad occurs is about 32% (10/31) XY cells. In the T16H/X *Sxr* animals that develop as females we propose that this critical proportion of cells with *Sxr* active was not reached. The hermaphrodites would be explained by an *Sxr* mosaicism in the region of threshold proportion, whereas the males of the same genotype would have proportions of cells with *Sxr* active above the critical levels. The relative proportions will vary from individual to individual, and even between the two gonads within a single individual, leading to unilateral hermaphroditism.

If the extent of spreading is linked to the primary X-inactivation event, the distribution of *Sxr* mosaicism among individuals will reflect the small number of cells present when inactivation occurs. The number of progenitor cells present in the embryo at this time has been estimated to be between 13 and 64[17]. Assuming a progenitor cell pool of at least 13 cells, and a requirement of about 30% gonadal cells with *Sxr* active over the period of testis determination for normal male development, a calculation based on binomial expectations suggests that inactivation would need to spread into *Sxr* in more than half the inactive X chromosomes to account for the observed number of hermaphrodites and females among T16H/X *Sxr* individuals.

Our observation of adult T16H/X *Sxr* hermaphrodites is consistent with earlier reports that X/X *Sxr* germ cells occasionally entered meiosis before birth[18], sometimes in association

Table 2 Progeny of T16H *Pgk-1*^{b}/+*Pgk-1*^{a} ♀ × X/Y *Sxr* ♂ matings

	Chromosome from male: X	X *Sxr*	Y	Y *Sxr*	Total
Chromosome from female (X) T16H *Pgk-1*^{b}	18♀	2♀ 5♂ 3⚥	36♂		64
+*Pgk-1*^{a}	23♀	13♂	24♂	11♂	71
Recombinant	2♀	2♂	3♂		7
Total	43	25	74		142

The progeny of the different types of *Sxr* ♂ did not differ significantly from one another and have been combined. The eight non-recombinant classes are expected to be produced in equal proportions.

Table 3 Progeny of T16H *Pgk-1*^{b}/+*Pgk-1*^{a} *Sxr* ♀

		From ♂*	
		X	Y
From ♀	T16H *Pgk-1*^{b} *Sxr*	5♂	10♂
	T16H *Pgk-1*^{b}	5♀	
	X *Pgk-1*^{a} *Sxr*	2♂†	5♂
	X *Pgk-1*^{a}	6♀	
	Recombinant	—	1♂

* A *Pgk-1*^{b} *Gs*/Y male was used to sire the first three litters, and a *Pgk-1*^{b} *Ta*/Y male for subsequent litters. Both males were extensively tested with normal females to ensure that they were not carrying *Sxr*.

† Phenotypically variegated coats.

with disturbed seminiferous tubule development[19]. A small proportion of the meiotic germ cells survived birth, to give rise to growing oocytes within the tubules, but no oocytes were seen in 30 adult X/X *Sxr* testes[20]. On their own, these earlier observations of ovotestes in young X/X *Sxr* animals could reflect merely some inadequacy in the masculinizing influence of *Sxr*, and/or the presence of two X chromosomes in the germ cells; but in the light of the present results on T16H/X *Sxr* animals, the earlier findings too can be taken to indicate that *Sxr* may be subject to X inactivation, even in X/X *Sxr* individuals[6]. The proportion of cells with *Sxr* inactivated by spreading will of course be lower in X/X *Sxr* than in T16H/X *Sxr* individuals, since only about half the *Sxr*-carrying X chromosomes will be inactive in the former, but all or almost all in the latter. An X/X *Sxr* individual in which sufficiently few cells expressed the male-determining *Sxr* sequences to allow the development of a fertile female would therefore be very rare indeed.

Two recently formulated sex chromosome models include the prediction that X-chromosome material in an embryo will be protected from inactivation if it has been paired during the preceding male meiosis[5,21]. Burgoyne[5] has accordingly suggested an explanation for the variable inactivation of *Sxr* when it is attached to an inactive X chromosome, namely that the expression of *Sxr* may be associated with a folding back and pairing of the *Sxr* region with the homologous testis-determining region in the same Y chromosome during the preceding male meiosis. If such pairing has occurred during meiosis, the *Sxr* region would be protected from subsequent inactivation in the embryo. To account for the observed hermaphroditism, one could further postulate that multiple male-determining sequences exist and that partial intra-chromosomal Y pairing may be followed by inactivation of a variable number of these sequences.

We did not know whether a T16H/X *Sxr* female, if she existed, would be fertile or sterile. The inactive X chromosome in female germ cells of the mouse is known to be reactivated at or shortly before entry of the germ cells into meiotic prophase[7,22–24]. In oocytes, therefore, both X chromosomes are expressed. The finding that female *Sxr* carriers can be fully fertile implies that the presence in female germ cells of male-determining DNA sequences on an active X chromosome from the time of entry into meiosis onwards does not impede the progress of the germ cells through oogenesis. This conclusion is consistent with a report[13] of a fertile female XX↔XY mouse chimaera that produced a functional XY egg, giving rise after fertilization to an XXY embryo. In another such chimaera, a single XY oocyte in first meiotic metaphase was identified cytologically[25]. A contrary conclusion, namely that the presence of *Sxr* prevents XX germ cells from undergoing oogenesis, was reached by Gordon[26] on the basis of a series of experimental chimaeras thought to have an X/X *Sxr* component. The evidence rests on a single female chimaera that produced germ cells from the non-*Sxr* component only; however, as the author himself points out, this could have been brought about by chance alone. Probably the chimaeras that involved an X/X *Sxr* component developed as phenotypic males.

In a recent study[27], *Drosophila* homozygous for mutant autosomal loci involved in sex determination developed sterile gonads devoid of germ cells. However, when the germ cells were introduced by pole cell transplantation into a normal embryo of the appropriate sex, they gave rise to normal functional gametes, suggesting that the mutant sex-determining loci did not affect development in the germ cells themselves. Our finding that the presence of *Sxr* in female germ cells does not interfere with oogenesis suggests that the same situation prevails in mice.

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Synchronized bursting of CA1 hippocampal pyramidal cells in the absence of synaptic transmission

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The synchronization of neuronal firing, seen at its most dramatic in the epilepsies, has generally been attributed to synaptic interactions[1–5]. We have now discovered a rhythmic spontaneous bursting activity produced by non-synaptic mechanisms. It develops in rat hippocampal slices after chemical synaptic transmission has been blocked by incubation in low Ca^{2+}, increased Mg^{2+} solutions, and persists with almost clockwork regularity for several hours. We report here that spontaneous firing of all the neurones recorded in the slice increased, consistent with the known effects of Ca^{2+} on membrane properties and synaptic transmission[6–10], but the synchronous 'field bursts', and presumably their underlying mechanisms, were restricted to the population of pyramidal neurones in the hippocampal CA1 region. Thus, these low Ca^{2+} field bursts are different from the Ca^{2+}-dependent synchronous bursts induced in slices by penicillin which originate in the population of pyramidal cells of the CA3 region[1–3].

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While the range of conditions that can elicit these field bursts remains to be established, it is particularly interesting that Ca^{2+} *in vivo* can drop to low levels, and starts to fall before the onset of seizures induced by pentylene tetrazol[11]. The precise roles in the field burst of non-synaptic mechanisms such as ephaptic interactions and K^+ movements have yet to be determined. The interburst interval, but not the burst size or duration, has proved particularly sensitive to manipulation of the Na^+–K^+ pump and also to a variety of drugs and transmitters in nano- to micromolar concentrations.

The methods of cutting and maintaining brain slices in our laboratories have been described previously[12–14] (see Fig. 1 legend). Although incubation of hippocampal slices in low (0.2 mM) Ca^{2+}, high (up to 5 mM) Mg^{2+} solution blocked synaptic responses completely after about 5–10 min, the bursting behaviour described here developed after much more prolonged exposure, typically 1–2 h, and persisted for over 12 h. It was reversed on returning to 2 mM Ca^{2+} solution. At first the bursts consisted of small groups of rather irregular population discharges which grew into mature 'field bursts' (see Fig. 1). These bursts lasted ~4 s and consisted of a large number of population spikes, each of which represented the synchronous discharge of many pyramidal cells, superimposed on a negative potential shift. The field bursts recurred with remarkable regularity, in this case every 21.6 s with a standard deviation (s.d.) of 1.6 s, with little variation in amplitude (mean, excluding spikes, 5.41 mV, s.d. 0.08 mV) or duration (mean 4.32 s, s.d. 0.34 s) during 45 min. Overall interburst intervals ranged from 15 s to several min, the amplitude up to 12 mV, and the burst duration from 0.5 to 20 s. The field bursts occurred synchronously throughout the CA1 in each of 48 investigated slices (12 rats). The two recording sites in Fig. 1*a*, *b* were 1 mm apart in the CA1 pyramidal layer. The bursts were closely synchronized at these and all other recording sites. No single site consistently discharged in advance of the rest of CA1, thus there is no evidence for a group of 'pacemaker' pyramidal cells. Incubation in the low Ca^{2+} solutions also changed the antidromic response evoked in the CA1 pyramidal layer by stimulation of the alveus from a single population spike to a burst of 5–20 population spikes. These brief evoked bursts could often trigger a full field burst. The other pyramidal cells (CA3 and 4) and the granule cells in the slice exhibited no spontaneous or evoked periodic behaviour. Therefore, the present phenomenon differs from the bursting induced by penicillin and bicuculline which is Ca^{2+}-dependent, always involves CA3, and consists of briefer bursts[1–3].

To explore the cellular basis of the low Ca^{2+} field bursts, we impaled neurones while the slices were bathed in normal Ca^{2+} solutions and held them during the change to low Ca^{2+} and the subsequent development of the bursts. The technical difficulty of this procedure has restricted the number of observations to four, but the comparisons are particularly reliable as they avoid problems of interneuronal variation and sampling errors. Results consistent with these prolonged recordings were obtained from a further 19 cells impaled while in low Ca^{2+}. Exposure to low Ca^{2+} increased the input resistance by 25% if the resting potential were held to its initial value by current injection. Otherwise the slowly developing ~20 mV depolarization was associated with an unchanged or reduced resistance. There was no detectable change in the threshold voltage for action potentials.

The intracellular data suggest that both the slow depolarization of the resting potential and the increased spontaneous discharge (from ~1 s^{-1} to 10 s^{-1}) following the change to low Ca^{2+} solution resulted from the block of inhibition by spontaneously active interneurones and from the depression of the afterhyperpolarization [gK(Ca)][7]. It is well known that reducing the concentration of divalent cations bathing a variety of nerve and muscle preparations increases excitability[6–9], probably through their effect on surface charge density. Mg^{2+} is less effective than Ca^{2+} in this role, thus the 0.2 mM Ca^{2+}, 4 mM Mg^{2+} solution used in most of the present experiments would be expected to have a net excitatory effect. The increased excitability may be an essential requirement for field bursts because they were abolished by raising Mg^{2+} to 6 mM, which counterbalances the excitatory effect of the low Ca^{2+} more closely[6]. However, we did not detect any change in the action potential threshold, which would be expected if the hyperexcitability were primarily due to deficiency of divalent cations. The conduction block and irreversible damage of neurones caused by Ca^{2+}-free solutions[6,10] were not seen here and would not be expected with the moderate changes in divalent cations used.

The potential shift recorded during the field bursts consisted of a 10–15 mV depolarization of the pyramidal cell membrane; its field was most negative in the cell body layer, and was positive in the outer two-thirds of the apical dendrites. This is consistent with a depolarization due to K^+, released by the excessive neuronal discharge, accumulating in the laminae around the pyramidal cell bodies. Using ion-sensitive electrodes, we have measured increases in K^+ activity in the pyramidal layer during, but not preceding, the bursts (three experiments). The peak K^+ activity reached 10 mM which approximates the ceiling of the physiological range[15]. If the

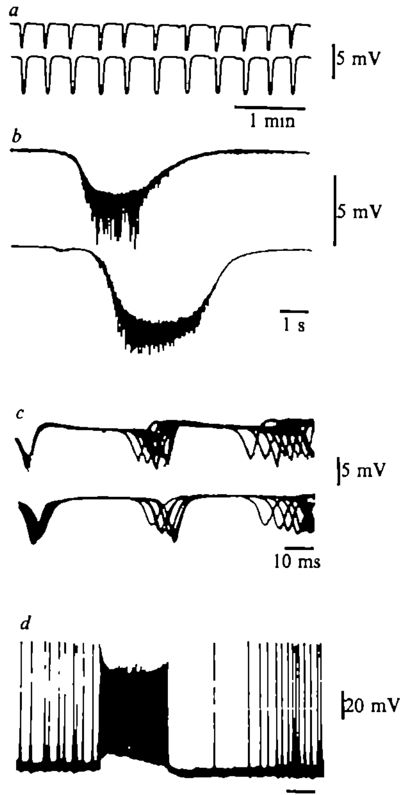

Fig. 1 Spontaneous 'field bursts' recorded from hippocampal slices which were incubated in 0.2 mM Ca^{2+}, 4 mM Mg^{2+}. The methods used have been described previously[12–14]. Briefly, 400 µm slices were prepared from rat hippocampus using a McIlwain tissue chopper or a Vibroslice and were incubated at 32 °C in a perfusion chamber in a thin film of perfusion liquid under warmed and moistened 95% O_2/5% CO_2. The standard media contained the following (in mM): Na^+, 150; K^+, 6.25, Cl^-, 137; Ca^{2+}, 2.0 or 0.2; Mg^{2+}, 2.0 or 4.0; HCO_3^-, 26; HPO_4^-, 1.25; glucose, 10; and were equilibrated with 95% O_2/5% CO_2 gas mixture to maintain the *p*H at 7.4. Micropipettes filled with 3 M NaCl (1–20 MΩ) or K-acetate (50–100 MΩ) were used for extra- and intracellular recording, respectively. *a*, Extracellular recordings from two sites, 1 mm apart, in the CA1 pyramidal layer. The negative field bursts recurred at 3 min^{-1}; *b*, their structure is shown in more detail on oscilloscope traces, population spikes descend from the negative potential shifts. *c*, Several superimposed sweeps during a field burst showing synchronization of population spikes. The recordings were made at sites 0.5 mm apart. *d*, Intracellular events corresponding to a field burst include a rapid, sustained burst of action potentials on a depolarizing shift, followed by a hyperpolarization.

resting potential were entirely due to K^+ this increase would correspond to a depolarization of 11 mV, which is close to the values we observed.

Treatments which depress the activity of ion pumps increased the frequency of the spontaneous bursts with little effect on their size or duration. Ouabain was effective at a concentration of 10^{-6} M; anoxia produced by reducing the gas flow over the slices also greatly speeded up the bursts (for example, from 0.2 min^{-1} to 10 min^{-1} within one minute of stopping the gas flow); cooling the slices from 32 to 25 °C significantly increased the burst frequency. A hyperpolarization was regularly recorded after the bursts, corresponding to a small, ouabain-sensitive, positive field in some extracellular recordings from the pyramidal layer, and may be attributed to the Na^+–K^+ pump. We postulate that sodium pump activity, stimulated by the increased extracellular K^+ (ref. 16), eventually terminates the burst by hyperpolarizing the cells for a period, and that the above treatments depress its ability to hyperpolarize without affecting its sensitivity to K^+.

Several drugs and transmitter candidates, applied in nano- to micromolar concentrations, modified the interburst interval but not the burst size or duration. The frequency of field bursts was increased by the excitatory amino acids glutamate, aspartate and DL-homocysteic acid, and by acetylcholine, noradrenaline, histamine and Li^+. The field bursts became less frequent during the application of the depressant amino acids γ-aminobutyric acid, taurine and imidazole acetic acid, and of serotonin, vasopressin and opiates. The stability of the phenomenon and its sensitivity to this wide range of drugs suggest that it may become a useful pharmacological tool (H.L.H. and J.G.R.J., in preparation).

The population spikes varied in amplitude during each field burst, producing characteristic shapes that varied between slices or even between different sites in CA1 of a single slice. However, our intracellular records showed a rapid and quite regular discharge. Thus for some periods during the field bursts, pyramidal neurones spread over substantial distances discharged in synchrony (Fig. 1*c*). The simplest hypothesis for the synchronization of the spikes is that extracellular currents, generated by pyramidal cells discharging, modulate the excitability of other members of the population, that is, 'field interaction'. Whether enough of the 'extracellular' loop of the action current passes through other cells to produce this effect depends on the detailed local geometry and electrical properties of the dendrites, glia and extracellular space. Experimental support for this possibility may be derived from the sensitivity of the smaller hippocampal granule cell to extrinsic electric fields[13]. The steady depolarization of the pyramidal cells in low Ca^{2+} would tend to increase the effectiveness of field interactions. The synchrony does not arise in the tightly-packed CA1 axons in the alveus as alvear fibre potentials were only seen after, and not preceding, the population spikes.

The synchronization of the bursts across the CA1 region remains to be explained. Paired recordings at sites 0.5–2.0 mm apart show that the leading site may vary between bursts. The latencies observed were up to 1 s, but more often ~50 ms. This is too fast to be explained solely by potassium movements of the kind involved in spreading depression, which propagates at a few mm per min[17]. We observed longer latencies, of the order of several seconds, between sites separated by an alvear lesion, so K^+ may transmit the field burst across these lesions. This also suggests that interactions within, or at least involving, the alveus contribute to the closer synchrony seen in the intact slice; but such interactions are not sufficient for synchronization, as lesions sparing the alveus alone result in two independent burst generators. Perhaps K^+ released by a bursting region can facilitate bursts in distant CA1 pyramidal cells more quickly by affecting their axons in the alveus than is possible through diffusion to cell bodies.

Alternatively, the burst synchronization, and even its generation, could be the result of specialized electrical contacts between the pyramidal neurones in a manner similar to that proposed for recurrent excitatory chemical synapses in CA3 (ref. 1). Electrical synapses are sensitive to the removal of divalent cations[10], although the small net changes involved in the present work might, if anything, be expected to weaken them. The existence of such contacts in CA1 is still controversial[18,19] and even if they were involved here, we would still have to explain why the bursts do not occur in CA3 where similar connections have been described.

Further work is needed to determine whether the mechanisms responsible for the present phenomenon operate *in vivo*. While low Ca^{2+} may not be the only route to their expression, and one might speculate about anoxia or metabolic defects, it is of particular interest that similar conditions can prevail in the intact animal. Measurements with ion-selective electrodes have shown that Ca^{2+} levels fall before the start of seizures induced by pentylene tetrazol[11] and can drop to 0.5 mM. During these seizures K^+ increased to 7–8 mM, which would also have enhanced the mechanisms involved here.

Calvin has commented on the likelihood of there being a wide variety of bursting mechanisms available in the repertoire of neuronal properties[20]. Previous studies of spontaneous synchronous bursting, notably in the hippocampal slice, have emphasized the role of synaptic connections. The phenomenon described here demonstrates how, in the absence of synaptic mechanisms, an increased neuronal discharge can be synchronized into a strongly rhythmic bursting behaviour.

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Intracellular recorded synaptic antagonism in the rat dentate gyrus

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With the exception of acetylcholine at the motoneurone–Renshaw cell synapse, the excitatory chemical transmitters of the major pathways in the vertebrate brain are unknown[1]. However, on the basis of biochemical and electrophysiological experiments at the extracellular level, the excitatory acidic amino acids glutamate and aspartate have long been suggested as the most likely candidates for this role[2–4]. Unfortunately, difficulties with recording intracellularly from postsynaptic cells at many of these synapses and the lack of selective antagonists have curtailed the criteria by which the identification of glutamate and aspartate as transmitters can be explored. We have now made intracellular recordings from granule cells of the dentate gyrus *in vitro* and report that γ-D-glutamylglycine

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(γ-DGG) is an effective antagonist of the excitatory postsynaptic potential (e.p.s.p.) evoked by stimulation of the medial perforant path (PP). During this antagonism the reversal level of the e.p.s.p. remains the same and the passive membrane properties of the postsynaptic membrane are unaltered. Quantal analysis shows that the reduction in the e.p.s.p. amplitude can be accounted for almost entirely by a reduction in quantal size while the quantal content remains unchanged, indicating a direct reduction in the postsynaptic sensitivity to the endogenous transmitter. (±)2-Amino-5-phosphonovalerate (APV) proved to be ineffective as a blocker of the PP-evoked e.p.s.p. and *cis*-2,3-piperidinedicarboxylate (PDA) caused excitation. The selectivity of γ-DGG and APV against the depolarization induced by the iontophoretic application of *N*-methyl-D-aspartate (NMDA), quisqualate and kainate closely resembled that in spinal cord. This constitutes the first evidence from a readily identifiable synapse in support of the view that excitatory amino acid receptors of the quisqualate/kainate type are involved in synaptic transmission and offers further support for the suggestion that the endogenous transmitter is aspartate or glutamate.

It has been proposed that three types of pharmacologically distinct excitatory amino acid receptors can be distinguished in the spinal cord, classified according to their most potent agonists as NMDA, kainate and quisqualate[5]. Thus APV is the most specific and potent antagonist of NMDA receptors[5,6]. γ-DGG, as well as blocking NMDA receptors, antagonizes responses to kainate[5,7], whereas PDA antagonizes all three receptor types[5,8]. Using intracellular electrodes we have studied the effect of APV, γ-DGG and PDA on the synaptic excitation evoked by PP stimulation in the granule cells of the dentate gyrus[9] where there is strong evidence favouring glutamate as the endogenous transmitter[10–13].

Impaled neurones, in a standard hippocampal slice preparation (see Fig. 1 legend), were characterized as granule cells by their position in the granular layer of the dentate gyrus, by orthodromic activation from the PP and by antidromic invasion from the mossy fibres region of CA_3[14,15]. Experiments were carried out on only those granule cells whose resting membrane potential (< -60 mV) and membrane input resistance remained stable throughout the experiment. Stimulation of the PP evoked an e.p.s.p. at least 8–20 mV in amplitude yet still below the threshold for initiating action potentials. This may be explained in part by the marked decrease in membrane resistance ($46 \pm 3\%$, $n = 6$) shown to be associated with the peak of the e.p.s.p. by computing voltage–current plots off-line with a PDP 11/23 computer (Cambridge Electronic Design Ltd). The same computer program was used to compute the apparent reversal level of the e.p.s.p. (-14.5 ± 3.4 mV, $n = 4$) by extrapolation from voltage–current plots determined from changes in peak e.p.s.p. amplitude (located mathematically) produced by the intracellular injection of long pulses (200 ms) of depolarizing and hyperpolarizing current. The voltage–current plots showed no significant deviation from linearity indicative of attenuation in e.p.s.p. amplitude caused by significant changes in the time constant of the cell soma or principal dendrites, or by the intracellular injection of current or extracellular application of amino acids.

On 29 granule cells, the iontophoretic application of γ-DGG (25–275 nA) markedly and reversibly reduced the amplitude of the e.p.s.p. evoked by stimulation of the PP (Fig. 1). The mean reduction of the e.p.s.p. was 61% (range 38–80%) of control. The effect of γ-DGG was not associated with a change in the resting membrane potential. Computer-derived voltage–current plots showed the resting membrane input resistance to be unaltered as was the excitability of the cell, tested both by determining the voltage threshold required for antidromic invasion and by the direct injection of depolarizing current.

These results do not exclude the possibility that the antagonism could be due to a presynaptic action of γ-DGG. However, in 12 experiments in which the mass presynaptic fibre volley could be recorded by our intracellular electrode (Fig. 1), its amplitude and duration were unaltered by the application of γ-DGG, even at the highest iontophoretic currents used (275 nA).

Independent evidence in support of the view that the action of γ-DGG on the postsynaptic membrane is competitive, came from a more detailed analysis of the e.p.s.p. For example, in five of six cells, the voltage–current plots showed the apparent reversal potential of the e.p.s.p. to be unaltered by the iontophoretic application of γ-DGG, that is, no systematic alteration occurred in the point of intersection of the extrapolated voltage–current plots derived on and off the peak of the e.p.s.p. in the presence and absence of γ-DGG. In effect, the decline in amplitude of the peak e.p.s.p. was accompanied by an increase in membrane resistance. This continuous linear relationship between the amplitude of the e.p.s.p. and membrane resistance is one prediction of the current theory that the amplitude of the e.p.s.p. is determined by the summation of individual currents generated by the opening of a relatively constant number

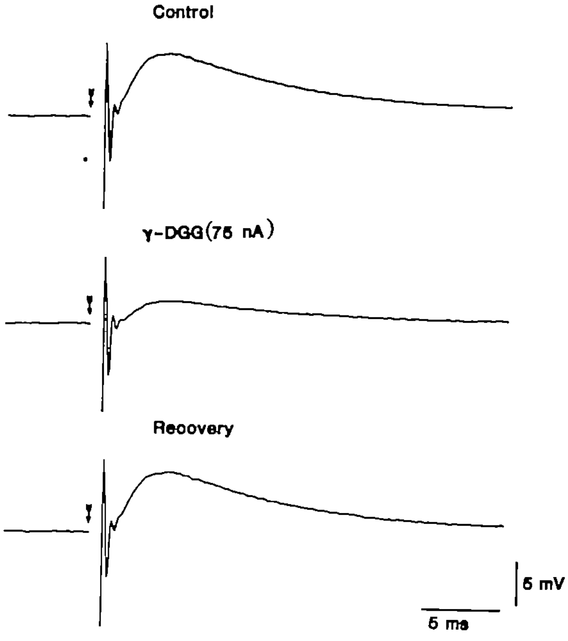

Fig. 1 Reversible reduction by γ-D-glutamylglycine (γ-DGG) of the medial perforant path-evoked e.p.s.p. recorded in a granule cell of the dentate gyrus *in vitro*. After impalement, the position of the iontophoretic electrode was adjusted along the dendritic tree at the level of the middle molecular layer until the response to a short (~1 s) application of glutamate was maximal. Glass micropipettes (tip diameter 3–6 μm) filled with 1 M NaCl were used as stimulating electrodes and positioned in the medial perforant path, 4–6 mm away from the impaled granule cell. Each trace is the computer average of 60 successive e.p.s.p.s recorded before (control), during (γ-DGG) and after (recovery) the iontophoretic application of 75 nA of γ-DGG. The e.p.s.p. was reversibly reduced from 8.5 to 3.2 mV. Our intracellular electrode recorded the presynaptic fibre volley which was unaltered by the application of γ-DGG. Arrows mark the preceeding stimulus artefact. Hippocampal slices (400-μm thick) were prepared from decapitated rats (200 g) using a McIlwain tissue chopper and maintained *in vitro* in a warm (36 °C) continuously oxygenated (95% O_2, 5% CO_2) medium containing (mM): NaCl 134, KCl 5, KH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 16 and glucose 10. Intracellular microelectrodes were filled with 1 M potassium acetate and had a resistance of 80–120 MΩ when measured *in situ* with a WPI model 707 electrometer. Drugs were applied by passing negative current through a multibarrelled micropipette containing L-glutamate (1 M, *p*H8), γ-DGG (0.2 M, *p*H8), APV (0.05 M in 100 mM NaCl *p*H8), PDA (0.2 M, *p*H8), NMDA (0.02 M in 150 mM NaCl, *p*H8), quisqualate (0.02 M in 150 mM NaCl, *p*H8), kainate (0.02 M in 150 mM NaCl, *p*H8) and NaCl (1 M, *p*H8). A positive backing current of 1–20 nA was applied when necessary.

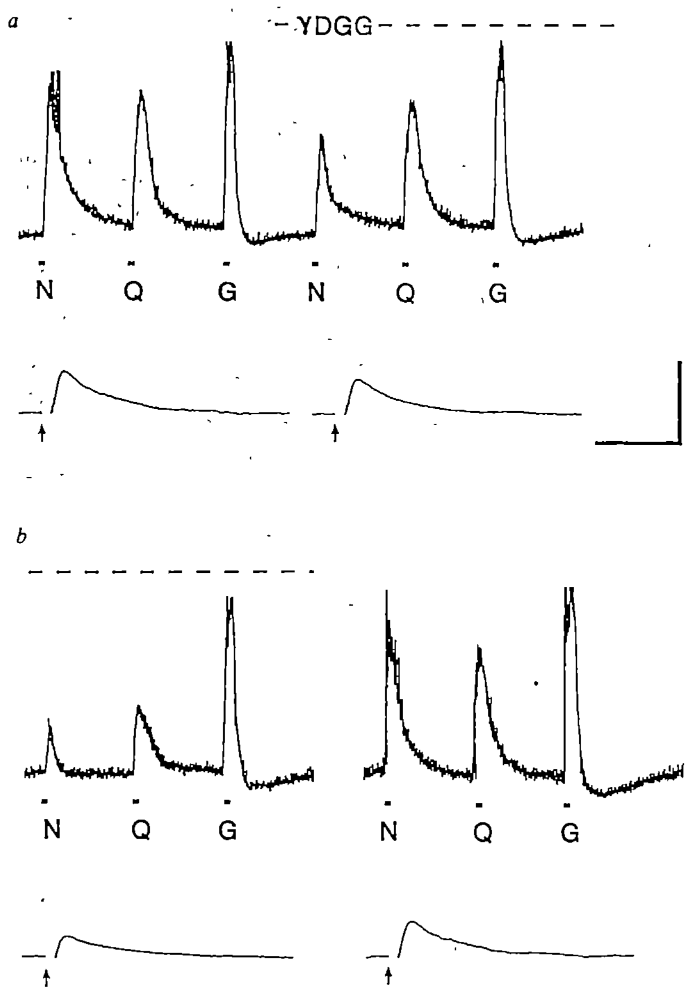

Fig. 2 Intracellular voltage recordings from a granule cell showing the effect of γ-DGG on the medial PP-evoked e.p.s.p. and the depolarization induced by pulsed iontophoretic applications of NMDA (N), quisqualate (Q) and glutamate (G). The upper trace is the intracellular voltage response to drug application, and the lower trace the computer average of 10 e.p.s.p.s. In *a* a 13-s application of γ-DGG was sufficient to reduce the NMDA-induced depolarization to 59% of control, while the e.p.s.p. was unaltered. In *b* the continuing application of γ-DGG reduced the response to quisqualate and the e.p.s.p. amplitude decreased from 11 to 5 mV. *a* and *b* are consecutive records and the gap in *b* is 1.5 min. The action potentials evoked by the drug-induced depolarization have been attenuated by the computer plotting subroutine. The durations of the amino acid applications are indicated by the black bars below the voltage records. Drug ejection currents were (nA): NMDA 100, quisqualate 150, glutamate 200 and γ-DGG 150. Calibration bars represent 20 mV and 30 s for the upper trace, 20 mV and 32 ms for the lower one. Solid arrows mark stimulation of the PP.

of transmitter-specific channels[16], and that antagonists simply combine with specific receptors to reduce the number of channels available. As reported below (Fig. 2) we also tested a further prediction of this theory by exploring the possibility that the potential evoked by the exogenous application of the transmitter involves similar if not identical receptor-activated channels.

In four cells in which all the criteria necessary to carry out a quantal analysis by the variance method[17,18] on trains of e.p.s.ps recorded in the presence and absence of γ-DGG were met, the decrease (46%) in the e.p.s.p. amplitude could be accounted for almost entirely by a reduction in quantal size (control, 294 μV; γ-DGG, 133 μV; $n = 4$) while the quantal content remained unchanged (control, 46; γ-DGG, 50; $n = 4$)[19]. Thus, according to the quantal model of synaptic transmission[20], the effect of γ-DGG on the PP-evoked e.p.s.p. is explained by a decreased sensitivity of the postsynaptic membrane to the endogenous transmitter without any appreciable change in transmitter release.

APV, the most potent and selective NMDA-receptor antagonist in the spinal cord[5,6], was found to be inactive as an antagonist of the PP-evoked e.p.s.p. In eight of nine neurones on which γ-DGG reversibly reduced the e.p.s.p., the iontophoretic application of APV (50–300 nA) was ineffective. In the only granule cell in which it appeared to be active, the decrease in the e.p.s.p. amplitude was not associated with any change in membrane potential or resting input resistance.

PDA, tested with iontophoretic current ranging from 1 to 50 nA, evoked an excitation of granule cells (six of eight neurones) accompanied by a depolarization and an increase in membrane resistance. Even at low iontophoretic currents, PDA produced burst-like activity.

The receptor and channels antagonized by γ-DGG, were characterized by comparing the action of γ-DGG on the PP-evoked e.p.s.p. with the amino acid-induced depolarization of granule cells. As shown in Fig. 2, the reduction in the e.p.s.p. amplitude only occurred when the application of γ-DGG was of sufficient magnitude to reduce the depolarization evoked by quisqualate and/or kainate, even though much smaller applications could block the response produced by NMDA. In the same experiments, we confirmed the even greater selectivity of APV for the NMDA receptor and its inability to effectively block the response induced by quisqualate and kainate.

In another series of experiments, short applications of γ-DGG but not APV were shown to antagonize the PP-evoked e.p.s.p. and glutamate-induced depolarization. However, when the action of γ-DGG on glutamate- as opposed to aspartate-induced depolarization was examined, no clear pattern of selectivity was observed. This was at variance with previous results in the spinal cord[5,7], in fact, in all six granule cells tested, γ-DGG was unselective and reduced the action of both excitants.

The results presented here show that γ-DGG is a potent antagonist of the PP-evoked e.p.s.p. and acts directly on the postsynaptic membrane to decrease its sensitivity to the endogenous transmitter. The amino acid receptors involved at this synapse are of the quisqualate and/or kainate type.

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Cross-neutralization of two different trypanosome populations derived from a single organism

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Antigenic variation observed during the course of infections with *Trypanosoma brucei* in mammals is caused by amino acid sequence changes in a variable surface glycoprotein (VSG)[1-3]. Antisera to isolated VSGs generally react only with the surface of homologous trypanosomes in neutralization and immunofluorescence tests[1,4], except in the case of certain 'isotypic' populations derived from different field isolates of trypanosomes[5]. An important question in evaluating the possibility of a vaccine is whether a single trypanosome can change into a second population which shares some, but not all, surface exposed epitopes during the limited time span of several sequential infections. If any trypanosomes in a mixture share exposed epitopes, the complexity of the necessary immunogen is reduced. We show here that two trypanosome clones WaTat 1.1 and WaTat 1.12, derived by differentiation from a single organism, have partially homologous VSGs and indeed share some similar exposed epitopes. Hence, a monospecific anti-VSG antiserum neutralizes both trypanosomes. However, several surface-exposed epitopes in WaTat 1.12 VSG exhibit reduced binding affinity for monoclonal antibodies made against WaTat 1.1 VSG and one epitope present on WaTat 1.1 trypanosomes is missing from the surface of WaTat 1.12 organisms.

A large number of trypanosome populations were derived from a single WaTat 1.1 organism as shown in Fig. 1*a*. The objective was to isolate from these populations trypanosomes which had similar exposed, surface epitopes. To do this, trypanosomes from each stabilized population were grown one passage in non-irradiated rats and live organisms analysed by immunofluorescence using rabbit antisera against WaTat 1.1 VSG. Anti-WaTat 1.1-reactive trypanosomes were present in many of the populations examined, but were generally less than 10% of the total population. However, in one population (from stabilate Rb8m7) there were 70% anti-WaTat 1.1-reactive trypanosomes. A clone (WaTat 1.12) was derived from this stabilate in lethally irradiated rats which reacted (>99% of the population) with rabbit anti-1.1 VSG sera. One rabbit antiserum against WaTat 1.1 VSG reacted with WaTat 1.12 trypanosomes to give non-uniform surface fluorescence (dots and lines) even though it reacted with the entire surface of WaTat 1.1 trypanosomes in the same staining conditions. The non-identity of these two trypanosome clones was confirmed by comparison of WaTat 1.1 and 1.12 isolated VSGs by column and analytical gel electrofocusing. On gel electrofocusing, the *p*Is of WaTat 1.1 and 1.12 VSGs were 8.5 and 7.0, respectively, relative to isoelectric point standards (Fig. 1*b*). WaTat 1.1 and 1.12 VSGs both had identical apparent molecular weights of 61,000 on SDS-gel electrophoresis (data not shown), a similar value to those obtained previously for other *T. brucei* VSGs[1,4,5].

Tryptic peptides of WaTat 1.1 and 1.12 VSGs were separated by two-dimensional chromatography–electrophoresis on TLC plates and their positions revealed by fluorescamine staining and examination under UV light (Fig. 2*a*). Complex maps were generated containing about 60 peptides, reflecting the high proportion of lysine residues present in *T. brucei* VSGs[1]. Clearly, many, but not all, of the peptides from the two VSGs were overlapping. This result was confirmed by a second peptide mapping procedure, autoradiographic analysis of tryptic peptides from ^{125}I-labelled VSGs. The effect of this procedure was

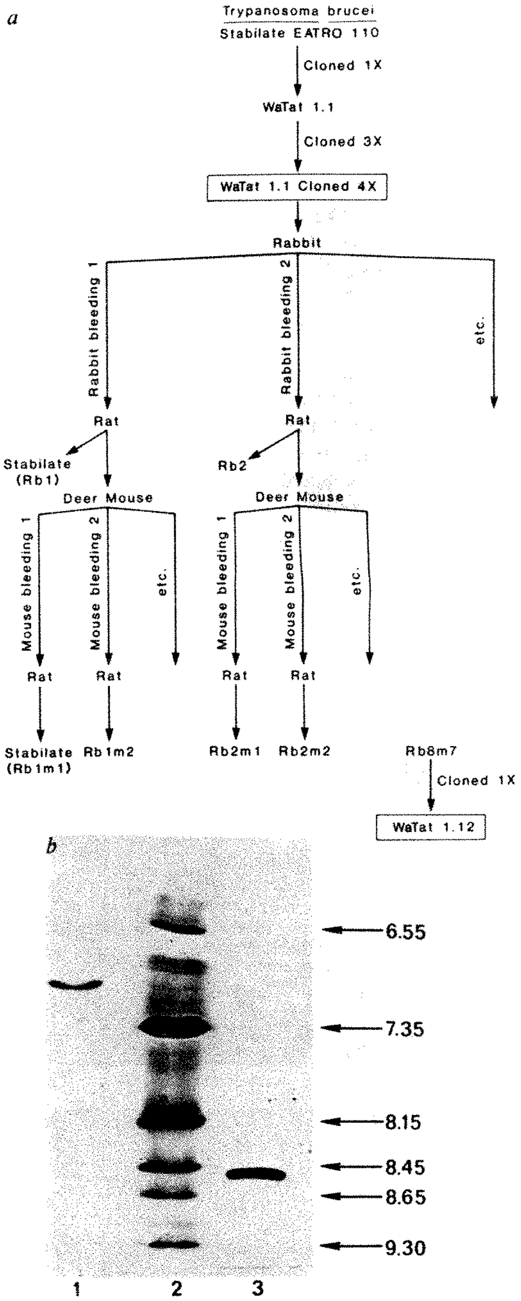

Fig. 1 *a*, Derivation of trypanosomes. Clone WaTat 1.1 was prepared from an EATRO 110 (East African Trypanosomiasis Research Organization, Tororo, Uganda) stabilate by injecting a single organism into the peritoneal cavity of a lethally irradiated (900R) rat. To ensure that all trypanosomes were derived from a single organism, WaTat 1.1 organisms were recloned three further times. VSG from all WaTat 1.1 populations had identical isoelectric points; also, rabbit antisera prepared against each WaTat 1.1 VSG[4] reacted in immunofluorescence with all WaTat 1.1 cloned populations. A rabbit was then infected with WaTat 1.1 trypanosomes (cloned four times) and blood taken from the ear at weekly intervals. Each sample of infected rabbit blood was inoculated into a non-irradiated rat and when the infection became patent stabilates were prepared; 0.2 ml of infected rat blood was also inoculated into a deer mouse (*Peromyscus maniculatus*). Blood was then taken at weekly intervals from the tails of infected deer mice, injected into non-irradiated rats and more stabilates made. A total of 103 stabilates were prepared, all of which consisted of equal volumes of infected rat blood and 15% v/v glycerol in PSG stored in liquid nitrogen. Clone WaTat 1.12 was derived from stabilate Rb8m7. The stabilate Rb8m7 was prepared about 4 months after the rabbit was infected with WaTat 1.1 trypanosomes. *b*, Analytical isoelectrofocusing of purified WaTat 1.1 and WaTat 1.12 VSGs in polyacrylamide gel at 4 °C. Coomassie blue staining of: (1) WaTat 1.12 VSG; (2) Pharmacia isoelectric point standards; (3) WaTat 1.1 VSG.

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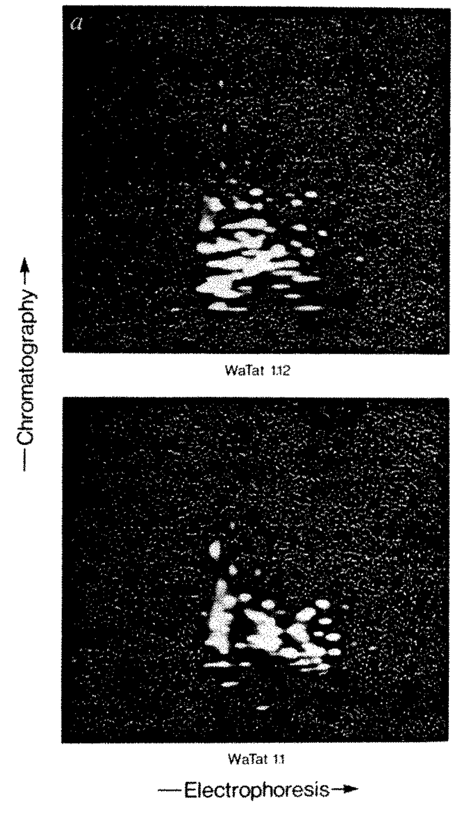
to reduce the map complexity but increase the specificity, because of the lower proportion of tyrosine compared with lysine residues in VSGs[1]. The ^{125}I-maps (Fig. 2*b*, *c*) again demonstrated similarity between WaTat 1.1 and 1.12 VSGs, but at least four out of the total 15–19 labelled tryptic peptides were dissimilar. As a control the peptide maps of WaTat 1.1 and 1.12 VSGs were compared to that of WaTat 1.2, a VSG from a third, randomly derived trypanosome clone but no similarities were observed (Fig. 2*b*, *c*). Thus these results agreed with the isoelectrofocusing data showing that WaTat 1.1 and WaTat 1.12 VSGs were not identical. The two VSGs did, however, share amino acid sequences which were not present in a third VSG.

Seven monoclonal antibodies were prepared against WaTat 1.1 VSG either by immunization of mice with isolated VSG or by immunization with irradiated (40,000R) trypanosomes. All seven antibodies bound ^{125}I-labelled WaTat 1.1 VSG but only five reacted in immunofluorescence with live WaTat 1.1 trypanosomes (Table 1). Therefore, not all the epitopes recognized in the isolated VSG were equally accessible in the same VSG on the surface of live trypanosomes. Of the five monoclonal antibodies which were reactive against WaTat 1.1 trypanosomes in immunofluorescence, four also reacted with WaTat 1.12 trypanosomes. One monoclonal antibody (Tryp 22A$_1$) reacted only with WaTat 1.1 trypanosomes, showing that an epitope exposed to antibody on WaTat 1.1 trypanosomes was not present on WaTat 1.12 trypanosomes. No evidence for population heterogeneity was obtained with these monoclonal antibodies, that is, when immunofluorescence reaction occurred, greater than 99% of the trypanosomes in that population reacted. Similarly, monospecific rabbit antisera against WaTat 1.12 VSG reacted with greater than 99% of both WaTat 1.1 and 1.12 trypanosomes.

As some, but not all, exposed epitopes were shared between WaTat 1.1 and 1.12 trypanosomes, we investigated whether cross-neutralization would occur. Results in Table 1 demonstrate that rabbit antiserum to either WaTat 1.1 or WaTat 1.12 VSGs neutralized both WaTat 1.1 and 1.12 trypanosomes and had no effect on WaTat 1.2 organisms. The two control sera (anti-WaTat 1.2 VSG and normal rabbit serum) were without effect on WaTat 1.1 or 1.12 trypanosomes.

We next analysed immunological cross-reactions between isolated WaTat 1.1 and 1.12 VSGs. Previous data have shown that homologous reactions between VSGs and monospecific rabbit anti-VSG sera were not effectively inhibited by heterologous VSGs[4] except in the case of 'isotypic' trypanosomes[5]. In radioimmunoassay, the homologous reactions ^{125}I-WaTat 1.1 VSG versus rabbit anti-WaTat 1.1 VSG serum or ^{125}I-WaTat 1.12 VSG versus rabbit anti-WaTat 1.12 VSG serum were effectively inhibited by both WaTat 1.1 and 1.12



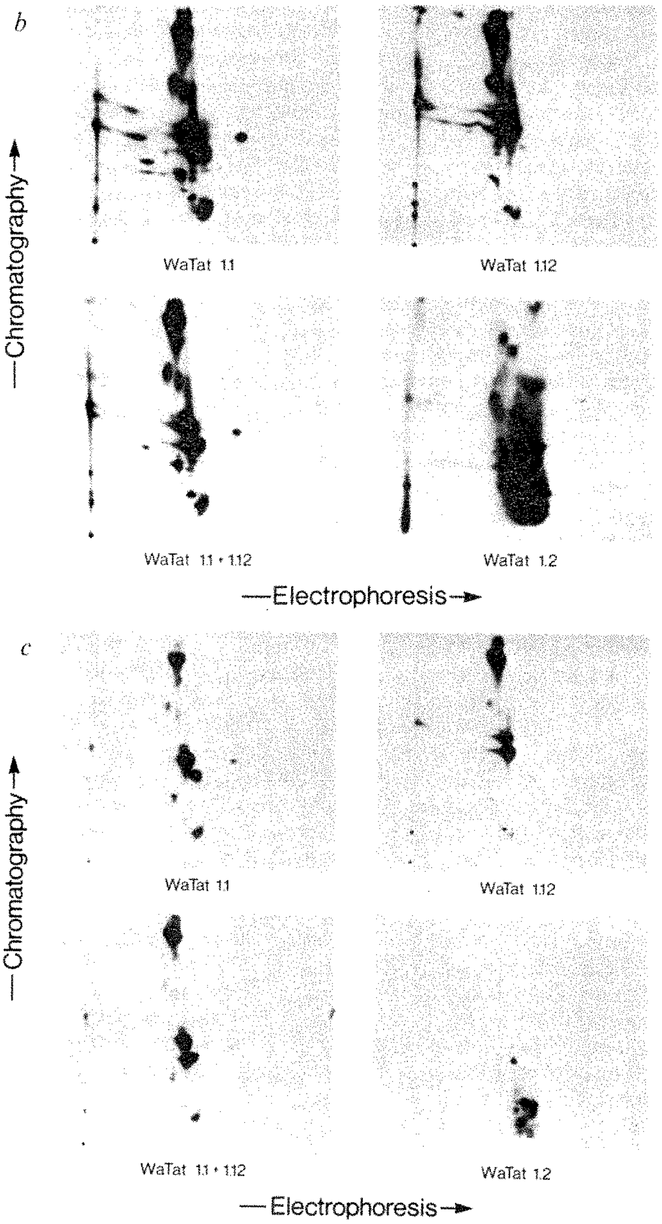


Fig. 2 Tryptic peptide maps of WaTat 1.1, WaTat 1.2 and WaTat 1.12 VSGs. Unlabelled VSGs (*a*) or VSGs labelled with ^{125}I using chloramine-T (*b*, *c*) were reduced with dithiothreitol, alkylated with iodoacetamide, precipitated with trichloroacetic acid and digested with trypsin (Sigma type XI, DPCC-treated) essentially as described in ref. 5 except that tryptic digestions were performed using 4% by weight trypsin: VSG for 17 h at 37 °C. Two-dimensional peptide mapping by thin layer electrophoresis and chromatography[19] was performed on cellulose plates. Electrophoresis was at 900 V for 70 min using as buffer, pyridine : acetic acid : water (1 : 10 : 89) *p*H 3.5. The chromatography solvent was pyridine : acetic acid : water : butanol (25 : 7.5 : 30 : 37.5). The position of peptides was revealed either by spraying with 0.01% w/v fluorescamine in acetone and photography under UV light[19] (*a*) or by autoradiography (*b*, *c*); *c* is an underexposure of *b*.

Table 1 Reactivity of antibody with different trypanosomes and VSGs

Antibody	WaTat 1.1 RIA	WaTat 1.1 FA	WaTat 1.1 N	WaTat 1.12 RIA	WaTat 1.12 FA	WaTat 1.12 N	WaTat 1.2 RIA	WaTat 1.2 FA	WaTat 1.2 N
Tryp 1E$_1$, monoclonal	+	+	ND	+	+	ND	−	−	ND
Tryp 2B$_1$, monoclonal	+	+	ND	+	+	ND	−	−	ND
Tryp 4C$_1$, monoclonal	+	+	ND	+	+	ND	−	−	ND
Tryp 22A$_1$, monoclonal	+	+	ND	−	−	ND	−	−	ND
WaT 8A$_1$, monoclonal	+	−	ND	+	−	ND	−	−	ND
WaT 20A$_1$, monoclonal	+	−	ND	+	−	ND	−	−	ND
WaT 24A$_1$, monoclonal	+	+	ND	+	+	ND	−	−	ND
Rabbit anti-WaTat 1.1 VSG	+	+	0/10	+	+	1/10	+	−	10/10
Rabbit anti-WaTat 1.12 VSG	+	+	0/10	+	+	0/10	+	−	10/10
Rabbit anti-WaTat 1.2 VSG	+	−	9/9	+	−	8/10	+	+	0/9
Normal rabbit serum	−	−	10/10	−	−	9/10	−	−	10/10

Monospecific rabbit anti-VSG sera were prepared as in ref. 4. Mouse monoclonal antibodies were prepared as described[20,21] using the SP2/0-Ag 14 myeloma cell line. The spleen cells for fusion were from mice immunized either with irradiated (40,000 R) WaTat 1.1 trypanosomes (Tryp series) or purified WaTat 1.1 VSG (WaT series). All hybridomas were double cloned. Radioimmunoassay (RIA) was as in Fig. 3 measuring binding of purified, ^{125}I-labelled VSGs to antibody. Immunofluorescence (FA) procedures were similar to those of ref. 4; the fluorescence of living trypanosomes was recorded immediately after the last wash. Neutralization (N) experiments were performed as follows. Ten trypanosomes in 50 μl of PSG (57mM Na_2HPO_4, 3mM NaH_2PO_4, 45mM NaCl, 55mM glucose, *p*H 8.0) were incubated with 100 μl of rabbit antiserum (inactivated at 56 °C for 30 min) for 30 min on ice and then injected into the peritoneal cavity of a mouse. Parasitaemia was observed by sampling tail blood at 2-day intervals. Numbers represent number of mice dead/total injected; all deaths were the result of trypanosome infection.

unlabelled VSGs. Inhibition of 100%, however, was only achieved by the homologous antigen. The heterologous antigen inhibited by a maximum of 70–90% (data not shown). A reaction of partial identity was also obtained between WaTat 1.1 and 1.12 VSGs in immunodiffusion.

The seven monoclonal antibodies made to WaTat 1.1 VSG were tested for binding to ^{125}I-labelled VSGs (Table 1 and Fig. 3). Six out of the seven bound both WaTat 1.1 and 1.12 VSGs. Monoclonal antibody Tryp 22A$_1$ only reacted with WaTat 1.1 VSG, a similar result to that obtained with the same antibody and live trypanosomes in immunofluorescence. Even though the remaining six monoclonal antibodies bound both WaTat 1.1 and 1.12 labelled VSGs, with four out of the six, more antibody was required to bind a given quantity of WaTat 1.12 VSG than 1.1 VSG. This indicated reduced binding constants of these four monoclonal antibodies for WaTat 1.12 VSG. The binding constant of antibody Tryp 4C$_1$ for WaTat 1.12 VSG was so low that binding was barely observable, even with undiluted antibody. None of the monoclonal antibodies bound ^{125}I-WaTat 1.2 VSG.

The data show that WaTat 1.1 and WaTat 1.12 trypanosomes: (1) are derived from a single trypanosome in a time period comparable with that available for variation in one host animal under field conditions; (2) express VSGs which are not identical in amino acid sequence, but do share regions of amino acid sequence homology not found in other, randomly isolated VSGs; (3) contain both similar and different surface-exposed VSG epitopes. We consider that the most likely mechanisms by which WaTat 1.1 trypanosomes differentiated into WaTat 1.12 are the following: (i) expression of alternative genes (perhaps members of the same VSG gene family[6,7]) present in WaTat 1.1 organisms; (ii) re-expression of the same WaTat 1.1 VSG gene which has undergone rearrangement[8] or mutation[9,10] either before or during re-expression; (iii) a combination of both mechanisms (i) and (ii), that is, expression of alternative genes and rearrangement or mutation. It may be possible to discriminate between these possibilities by analysing for the presence of WaTat 1.1 and 1.12 genes in both trypanosomes.

It might be argued that oligosaccharides could be responsible for the cross-reactions observed between WaTat 1.1 and WaTat 1.12 VSGs. We do not consider this likely because: (1) oxidation of WaTat 1.1 VSG with 100 mol periodate per mol VSG (48 h, 4 °C, as in ref. 11) destroyed binding of the antigen to rabbit anti-WaTat 1.2 VSG sera (due to cross-reacting oligosaccharide[11,12]), but did not affect binding of the oxidized WaTat 1.1 VSG to rabbit anti-WaTat 1.12 VSG sera; (2) various studies using periodic acid–Schiff staining and electron microscopy, immunochemical techniques and sequencing[11–17] suggest that VSG carbohydrate is on the innermost side of the surface coat next to the cytoplasmic membrane, and is not accessible for binding to lectins or antibody when on the trypanosome surface. The monoclonal antibodies shown in Fig. 3 react with surface-accessible epitopes.

These results have theoretical and practical implications. First, a single trypanosome may vary during a short time period into a population expressing a different VSG, from which it might not be distinguished using monospecific or even some monoclonal anti-VSG sera in immunofluorescence. We suggest that amino acid sequence identity between expressed VSGs be the ultimate criterion of whether two trypanosome populations are identified as being similar. The data also provide the first steps to a classification of different trypanosome populations depending on shared, exposed epitopes present on their surface. This classification would be useful in deciding whether cross-immunization between any two trypanosome populations was possible.

Second, reappearance of the same antigenic type of trypanosome at different times during a cattle infection has been suggested previously[18]. This phenomenon could, clearly, now also be attributed to appearance of a trypanosome expressing a similar but not identical VSG.

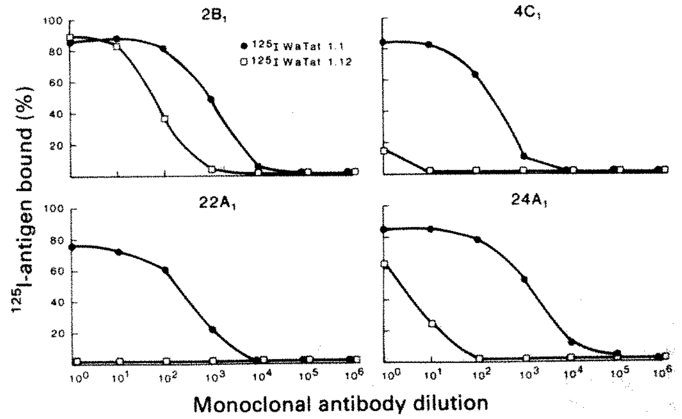


Fig. 3 Binding of monoclonal antibodies to ^{125}I-labelled WaTat 1.1 and WaTat 1.12 VSGs. Reaction mixtures contained 20 μl of ^{125}I-labelled VSGs[4] (5ng in all cases), 20 μl of monoclonal antibody and 20 μl of TEN buffer (0.02 M Tris-HCl, 0.005 M EDTA, 0.1 M NaCl, 0.015 M NaN_3, *p*H 7.6 and 5 mg ml^{-1} bovine serum albumin). The mixture was incubated for 1 h at room temperature then 100 μl of a 10% suspension of formalin-fixed *Staphylococcus aureus* (Pansorbin, Calbiochem) was added. The incubation continued for another hour before the mixture was centrifuged at 1,500*g* for 20 min, washed twice in TEN buffer and radioactivity in the final pellet measured. All dilutions were made in TEN buffer.

Finally, these two trypanosome populations represent the first available for analysing rearrangements and mutations in VSG genes and relating DNA sequence changes to modifications in individual, surface-exposed epitopes.

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A human suppressor T cell clone which recognizes an autologous helper T cell clone

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In contrast to the many reports of IL-2 dependent proliferating, helper or killer cell clones[1], there is only a single report of an IL-2 dependent suppressor cell clone[2], in the mouse. However by the same cloning procedures used to generate human helper cells[3], suppressor cell clones to influenza virus could not be generated, and so another strategy was used. Jerne's network hypothesis[4] proposes that immune regulation results from lymphoid cell receptors recognizing determinants on other lymphoid cell receptors. If this is the case it should be possible to generate regulatory T cell clones against other T cells and we report here the generation of an autologous suppressor cell clone which recognizes and inhibits the function of a human helper T cell clone. Such an autologous suppressor cell clone provides a new approach to understanding the pathways and molecular events involved in immune regulation. Mutual stimulation of the suppressor cell clone and the target helper cell clone in the absence of back stimulation, provides direct experimental evidence for the existence of interactions between T cell receptors, and thus suggests that the specificity of the suppressor cell clone is for the antigen receptor of the helper cell.

Human immunology, unlike mouse immunology, is limited by restricted access to genetically uniform cells. In order to circumvent this problem, all the experiments reported here were performed with cryopreserved cells from a single individual. T lymphocyte clone 6 (TLC 6) is a helper cell which recognized influenza A matrix protein in conjunction with HLA-DR1[3-6]. It was used to generate anti-idiotype suppressor cells because it grows well in culture, and its helper activity is easy to measure *in vitro* using the influenza virus specific antibody response and assay system developed by our colleagues, Callard, Beverley and Zanders[7-9], using either the cells themselves, or its antigen specific helper factor.

To stimulate autologous peripheral blood cells (PBL) with irradiated TLC6, a 10-day mixed lymphocyte culture was performed. The resulting blasts were purified on Ficoll–Hypaque and restimulated with irradiated TLC6. The ensuing lymphoblasts purified on a Percoll gradient three days later, and cloned as described in Table 1. Approximately 60 clones were grown up, and tested for their specificity. For simplicity, proliferation was used as the criterion of responsiveness, and cells from individual clones were cultured with irradiated autologous PBL and either the immunogen TLC6, another helper clone from the same donor HA1.9, influenza A virus, or just PBL alone. After 72 hours, cultures were pulsed with tritiated thymidine and cells were collected 16 hours later. Several patterns of reactivity were seen. Clones from these experiments were termed AC1 to 60. The most common response was to influenza virus (for example clone AC10), which could have been still present on the surface of washed TLC6 even after one week's culture. Other clones showed no detectable response to any of the stimuli used (for example AC57). This is paradoxical, as only activated cells respond to TCGF and can be cloned[1]. It seems likely that these clones were responding to TLC6, but the response was not detectable by the assay used. Clone AC34 is presumably recognizing an antigen on PBL (or serum), whereas clone AC5 is recognizing an activated cell antigen. The specificity of clones AC19 and AC22 is not known.

The most relevant clone, chosen for further analysis, was clone AC50. This proliferated in response to TLC6, but not other clones, PBL or influenza virus. Thus it seemed to be specific for TLC6, and was a candidate for an anti TLC6 clone. In the absence of any antibodies which unambiguously define human T-cell receptors, we used a functional assay to analyse the specificity of clone AC50 further.

We argued that if receptors on AC50 recognized anti-TLC6 receptors, then TLC6 might be stimulated to respond to irradiated AC50 *in vitro*, as its receptors would be bound. Thus a variety of influenza specific clones (all from the same donor) were stimulated with irradiated clone AC50, or AC50 plus irradiated E^- cells (T depleted cells) as a source of antigen presenting cells. As a control the response to AC50, PBL and influenza virus was assayed. As shown in Table 2, all the clones responded to influenza virus and TCGF, but only TLC6 responded to AC50, with or without the addition of irradiated E^- cells. This result is compatible with the notion that AC50 is an auto anti-idiotype clone, that is, recognizes antigen-specific receptors on TLC6. The response in the absence of additional antigen presenting cells (APC) is of interest, implying that the reaction between TLC6 and AC50 may not require antigen presenting cells. However, a few APC, which had not adhered to the plastic in the week of culture since irradiated PBL were added could still have been present, and this question needs to be analysed in detail. It is of interest that Infante *et al.*[10] recently found that anti-idiotype antisera stimulate mouse T cell clones to proliferate even in the absence of added antigen presenting cells, in analogy to the results shown here with anti-idiotype T cells.

The results in Table 2 indicate that AC50 and TLC6 interact in a specific manner. But they do not conclusively indicate that the reaction is anti-receptor. An alternative interpretation is that the proliferation of TLC6 is due to back stimulation[3,11] with irradiated AC50 recognizing TLC6, and it is the release of lymphokines from irradiated AC50 which causes TLC6 to incorporate thymidine. This possibility was investigated, using a cultured (not cloned) T lymphocyte line (CTLL) which responds well to TCGF (Table 3). A mixture of irradiated AC50

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Table 1 Induction of anti-TLC 6 clones

	Antigen					
Clone no.	6+PBL	HA1.9+PBL	PBL	Flu+PBL	Medium	TCGF
AC5	1,249±94	5,469±1,595	44±10	75±21	39±16	11,450±750
AC10	43±20	57±16	54±16	4,676±404	31±11	5,063±162
AC19	2,534±367	2,794±366	2,457±326	31±8	15±4	3,164±243
AC22	1,518±247	24±2	46±11	2,478±35	34±4	8,695±622
AC34	1,801±360	2,255±304	1,655±68	3,005±912	29±7	6,243±221
AC50	2,928±94	32±13	36±10	54±8	21±3	9,949±216
AC57	23±4	22±5	24±4	30±5	21±2	9,720±506
AC58	4,550±570	3,711±446	7,508±1,620	5,801±1,494	26±3	9,097±208

Peripheral blood lymphocytes (PBL, 5×10^5 per ml) were cultured with irradiated (2,500 rad, ^{137}Cs) TLC6 (5×10^5 per ml) in RPMI 1640 supplemented with 10% pooled A$^+$ serum in a 24 well plate (Costar). Both the responder (PBL) and stimulator (TLC6) cells were derived from the same donor. After 10 days of primary culture the viable cells were isolated on Ficoll–Hypaque and blast cells (10^5 per ml) were restimulated with irradiated TLC6 (10^5 per ml) in the presence of autologous irradiated PBL (5×10^5 per ml) Three days after restimulation the lymphoblasts were enriched on a discontinuous Percoll (Pharmacia) density gradient (35–40%) and plated at 0.3 cells per well in sterile 60 well Microtest II trays (Falcon) in the presence of T cells growth factor (TCGF or Interleukin 2) irradiated stimulator cells (TLC6; 5×10^3 per well) and autologous irradiated PBLs (5×10^3 per cell) as a source of antigen presenting cells (APCs) TCGF was prepared from PBL (1×10^6 per ml cultured with PHA (0.1%) in the presence of 1% autologous serum for 48 hours as described elsewhere[3,11]. The wells that showed positive growth (3.4% of the total number of blast cell seeded) at 7 days were transferred to 96 well flat bottom microtitre trays (Falcon) and cultured with irradiated TLC6 (5×10^4 per well) and autologous irradiated PBL (5×10^4 per well) in the presence of TCGF. Following a further 7 days in culture, the clones were transferred to 24 well trays containing TCGF, irradiated TLC6 (5×10^4 per well) and autologous irradiated PBL (1×10^6 per well). In the preliminary screen clones were examined for antigen specificity in a 72 hour array by using titrated methyl thymidine (^{3}H-TdR; NEN). A 1:200 dilution of individual clones from 24 well trays were cultured with autologous irradiated PBL (25×10^3 per well) and irradiated TLC6 (5×10^3 per well) in a total volume of 200 µl. Clones T cells from the same donor but with a different antigen specificity (HA1.9) together with autologous PBL, PBL alone or influenza A virus (A Texas/1/77; 5 haemagglutinating units (HAU) per ml) in the presence of PBL were added as controls. After 72 hours, cultures were pulsed with 1.0 µCi of ^{3}H-TdR and cells were collected 16 hours later. Incorporation of ^{3}H-TdR was measured by liquid scintillation spectroscopy. Results are expressed as mean c.p.m.±s.e.m. We have checked AC50 for its reactivity with other helper clones from the same individual but with different fine specificities. It did not react with 2 other helper clones (HA1.7, HA1.9) as well as other autologous clones (37, 53, 72).

and TLC6 could not stimulate CTLL to proliferate (31 c.p.m.) in contrast to TCGF (6,503 c.p.m.), as shown in Table 3. There was no effect of AC50 and TLC6 even in the presence of E- cells.

Thus the induction of thymidine incorporation in TLC6 by AC50 was not due to back stimulation, and thus the bidirectional nature of the TLC6–AC50 interaction and its specificity for TLC6, but no other autologous clones would appear to indicate that AC50 recognizes antigen-specific receptors on TLC6. Because the reaction is autologous, and specific for TLC6 it cannot be directed against MHC or differentiation antigens. The criteria we have used to define the anti-idiotype nature of clone AC50 (that is, specificity, stimulatory effects) are the same as used for anti-idiotypic antisera (discussed in refs 10, 12).

The effects of clone AC50 on the helper response of TLC6 were assessed *in vitro* (Table 4). Very small numbers of AC50 diminished the response. Only 10 clone AC50 cells inhibited the helper response of 500 TLC 6 (to 100,000 E$^-$ cells in a 200 µl culture volume) by 95%. Fifty cells of AC50 per culture abolished the response altogether. These results indicate that AC50 is a potent suppressor cell. The mechanism of suppression is unknown, but it is unlikely, due to the ratio of cells used (1 suppressor to 50 helpers) and the capacity of irradiated AC50 to induce TLC6 to incorporate thymidine (Table 2), that AC50

Table 2 Anti-idiotypic activity of clone AC50

	Antigen				
		Clone AC50*			
Clone no.	(5×10^2)	(1×10^3)	(5×10^3)	Medium	TCGF
6	820±124	1,160±169	2,274±322	17±4	3,472±313
6+E$^-$*	3,413±369	4,074±254	5,328±590	31±16	
6+E$^-$+Flu	10,311±2,404	8,902±866	7,386±1,063	9,360±495	
37	43±9	59±8	65±16	35±3	8,551±1137
37+E$^-$	38±8	71±18	32±4	53±13	
37+E$^-$+Flu	13,569±1,861	9,978±1,348	11,703±1,018	14,325±2,543	
53	27±4	35±12	17±6	25±6	6,217±491
53+E$^-$	33±5	18±2	24±2	12±1	
53+E$^-$+Flu	4,261±434	4,954±129	6,203±631	4,829±686	
72	23±1	8±2	19±5	20±5	4,142±474
72+E$^-$	41±14	26±3	13±2	29±3	
72+E$^-$+Flu	7,443±1035	7,248±131	8,130±1076	7,055±360	
HA1.7	17±2	15±2	10±3	31±6	3,447±122
HA1.7+E$^-$	33±1	43±8	18±2	12±1	
HA1.7+E$^-$+Flu	3,625±329	4,288±372	4,100±105	3,814±261	
CTLL	58	49±11	115±17	73±14	10,361±1,895
CTLL+E$^-$	101	146±23	742±109	60±8	
CTLL+E$^-$+Flu	20,183	17,081±3,036	15,622±1,388	18,762±2,573	

Various numbers of irradiated AC50 cells (5×10^2, 1×10^3 and 5×10^3 per well) were cultured with a panel of influenza A virus-specific TLCs (5×10^3 per well) in the presence or absence of autologous irradiated sheep erythrocyte (SRBC) rosette negative (E$^-$) cells (5×10^3 per well) and Influenza A virus. E$^-$ cells were separated from those cells forming rosettes with AET (aminoethylisoethiouronium bromide hydrobromide)-treated SRBC by centrifugation over Percoll (1,080 g ml^{-1}). Proliferative responses were determined by the incorporation of ^{3}HTdR as described in the legend to Table 1. Results are expressed as mean c.p.m. (±s.e.m.). CTLL, influenza specific T lymphocyte line.

* E$^-$ and AC50 irradiated 2,500 rad.

Table 3 Lack of back stimulation in mixed cultures of AC50 and TLC6

Responder cells	Stimulation (c.p.m. ± s.e.m.) AC50	6	AC50+6	FluA	Medium	TCGF
CTLL	43 ±7	17 ±4	31 ±9	15 ±2	23 ±6	6,503 ±294
CTLL+E^-	12 ±5	21 ±5	57 ±8	14,689 ±2,229	27 ±4	—

Irradiated AC50 (5×10^3 cells per well) and TLC6 (5×10^3 cells per well) were cultured alone or together with an influenza specific cultured T lymphocyte line (not the same as in Table 2) CTLL (5×10^3 cells per well) in the presence or absence of autologous irradiated E^- cells. As controls CTLL cells were cultured with and without irradiated E^- cells and influenza A virus. Proliferative responses were measured and results expressed as described in Table 1.

is cytotoxic for TLC6. The mechanism of suppression remains to be elucidated, but because it occurs at the clonal level should be amenable to precise analysis.

These results indicate that as in the mouse, it is possible to generate a human specific suppressor T cell clone dependent on IL-2. In contrast to the mouse suppressor cell clone, specific for antigen, the specificity of the human suppressor clone was for the immunizing cell (and not other autologous helpers). The reciprocal stimulation, in the absence of back stimulation, suggests that the target of AC50 is the receptor for antigen of TLC6. This is a direct experimental test of the 'idiotype network' at the T cell level. The potency of the suppressor effect suggests that this type of suppressor cell may be of major importance in immune regulation.

The generation of suppressor T cells recognizing helper cell receptors resembles work reported by Binz and Wigzell, who injected antigen-specific lymphoblasts into autologous mice[13]. Specific unresponsiveness resulted, which was mediated by either T killer, suppressor or helper cells, or B cells[13]. Conceptually the results are analogous, but the experiments reported here were performed entirely *in vitro*, and at the clonal rather than the population level. There are reports of suppressor cell pathways to the haptens nitrophenyl (NP) or azobenzene arsonate (ABA) for delayed hypersensitivity which involve second order suppressor cells, which recognize idiotype bearing cells[14]. However, the final suppression was not idiotype specific[15]. These experiments were also performed at the population level.

Table 4 Cloned auto (anti-idiotypic) suppressor T lymphocytes

	Stimulus		Suppression	Response
Helper T cells	Autol. E^- (5×10^5 per ml)	A/Texas/1/77 (0.5 HAU per ml)	(AC50 per ml)	Anti-influenza A virus antibody (ng ml^{-1})
E^{+*}	−	−	−	0
E^+	−	+	−	0
−	+	−	−	0
−	+	+	−	4±1
E^+	+	+	−	145±26
TLC6†	−	−	−	0
+	+	−	−	0
+	+	+	−	208±22
+	+	+	50	10±2
+	+	+	250	3±1
+	+	+	500	0

Cloned helper T cells (TLC6; 2.5×10^3 per ml) or E^+ cells (5×10^5 per ml) were cultured with autologous E^- cells in the presence of influenza A virus (A/Texas/1/77; 0.5 HAU ml^{-1}) in RPMI 1640 supplemented with 10% horse serum in 96 well round bottom microtitre trays. TLC AC50 cells were added at 50, 250 and 500 cells per ml at the initiation of the cocultures each of 200 µl. After 6 days incubation at 37 °C, triplicate cultures were washed and recultured in 150 µl of RPMI 1640 supplemented with 5% fetal calf serum (FCS). Supernatants were collected after 24 h and assayed for anti-A/Texas/1/77 antibody using a solid phase enzyme immunoassay. Antibody production was determined by reading absorbance at 405 nm of the transformed substrate of alkaline phosphatase[9]. The actual amount of antibody (ng ml^{-1}) ± standard error of triplicate cultures. Results are expressed as the mean (ng ml^{-1}) and for each triplicate the s.e. was <20%. Background responses of TLC6, E^+ and E^- cultured alone or with influenza A virus were measured. * 5×10^5 ml^{-1}; † 2.5×10^3 ml^{-1}.

By possessing a clone of suppressor cells (AC50), specific for a clone of helper cells (TLC6), we have a powerful system for analysing the mechanism of T cell suppression. There is no reason to believe that the principle demonstrated here will not apply with other clones. Thus, situations with undesirable clones of helper (or killer) cells such as autoimmunity or perhaps even leukaemia may be controllable by suppressor cells of this type, generated *in vitro*.

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A protein with kinase and phosphatase activities involved in regulation of tricarboxylic acid cycle

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Phosphorylation–dephosphorylation reactions as a form of regulatory control are assuming increasing importance in our understanding of cellular processes. The phosphorylation–dephosphorylation reaction was the first example of covalent modification in control and has been found to be present in almost every type of cellular process including synthesis of cell constituents, metabolic pathways and even gene expression[1]. It has assumed increasing importance as a result of the finding that some proteins coded for by carcinogenic viruses apparently are involved in a phosphorylation reaction[2,3]. To better understand the principles underlying these processes, we have studied the role of phosphorylation of isocitrate dehydrogenase (IDH) in the regulation of the branch point between the tricarboxylic acid (TCA) cycle and the glyoxylate bypass. We report here that in the process of purifying the kinase and phosphatase, we made two surprising observations—the kinase and phosphatase activities appear to be associated with the same protein, and the phosphatase activity has an absolute requirement for ATP.

During growth on acetate, *Escherichia coli* achieves net synthesis of cellular intermediates by induction of the glyoxylate bypass[4]. This anapleurotic pathway competes with the TCA cycle, the principal producer of metabolic energy, for a limiting amount of isocitrate. The branch point between these pathways

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has been shown to be regulated, at least in part, by phosphorylation of IDH[5-7].

Our first goal in the study of this branch point was to purify the relevant enzymes. We purified IDH kinase by using three steps: ammonium sulphate fractionation, chromatography on DEAE–Sephacel and affinity chromatography using IDH immobilized on Sepharose 4B. Absorption of the IDH kinase to the affinity column was found to require the non-hydrolysable ATP analogue, 5′-adenylylimidodiphosphate. After sample application, the column was washed with buffer containing the analogue until the effluent was free of protein. The bound enzyme was then eluted by washing the column with a buffer which did not contain the analogue. Figure 1 presents the results of electrophoretic analyses of the peak activity fractions; they each contain a single protein band, the intensity of which was proportional to the amount of IDH kinase activity. The mobility of the protein band indicates a molecular weight (MW) for the protein of 66,000.

We then proceeded to purify the IDH phosphatase. During preliminary experiments it became apparent that the steps needed for phosphatase purification paralleled those for the kinase and, indeed, that the ratios of the two activities remained almost constant. We therefore focused our attention on attempts to separate the two activities by various techniques. In our first attempt we used ion-exchange chromatography on DEAE–Sephacel (Fig. 2). This column yields good resolution of the applied protein, typically producing 8–12-fold purification of the IDH kinase. Despite this efficiency, the kinase and phosphatase activities eluted superimposably. Our next attempt to separate the two activities used a Sephadex G150 column, and again the activity peaks were indistinguishable. Finally, we re-examined our IDH–Sepharose affinity column and found that both the kinase and the phosphatase absorbed to the column in the presence of the ATP analogue and were superimposably eluted when the analogue was removed. The single protein band which eluted with the enzyme activity, as described above, showed a constant ratio of kinase to phosphatase activity. Thus, IDH kinase and IDH phosphatase are chromatographically indistinguishable when tested by ion-exchange, gel filtration and affinity column techniques. The purified activities exhibit a single protein band. Therefore, these activities appear to be associated with the same protein and possibly the same polypeptide chain. These observations do not, however, completely exclude the possibility that the two activities exist on highly similar proteins, or on non-identical subunits of a heterodimer.

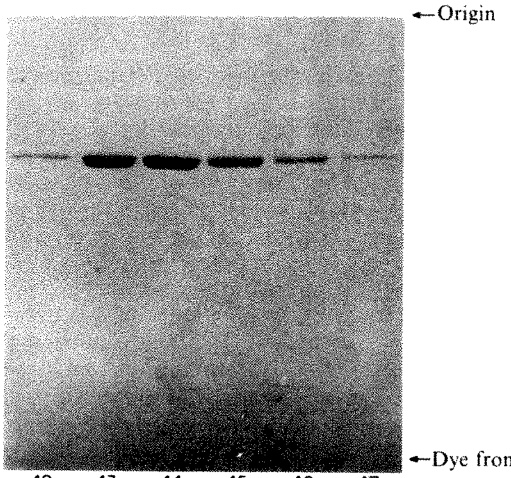

Fig. 1 SDS-polyacrylamide gel electrophoresis of purified IDH kinase following IDH–Sepharose chromatography. Partially purified IDH kinase was prepared by ammonium sulphate precipitation and DEAE–Sephacel chromatography. The sample, in buffer A (25 mM MOPS *p*H 7.0, 50 mM NaCl, 5 mM $MgCl_2$, 2 mM 2-mercaptoethanol, 0.5 mM 5′-adenylylimidodiphosphate), was applied to an affinity column prepared by immobilization of IDH on Sepharose 4B. The column was then washed with buffer A until the effluent was free of protein. The bound IDH kinase was then eluted by washing the column with 25 mM MOPS *p*H 7.0, 50 mM NaCl, 5 mM EDTA, 2 mM 2-mercaptoethanol. After assay for IDH kinase, samples from peak activity fractions were subjected to SDS-gel electrophoresis on 10% polyacrylamide gels. The gels were stained with Coomassie brilliant blue and destained with 10% acetic acid. Molecular weight standards, electrophoresed simultaneously, were phosphorylase *b* (94,000), bovine serum albumin, BSA (67,000), ovalbumin (43,000) and carbonic anhydrase (30,000).

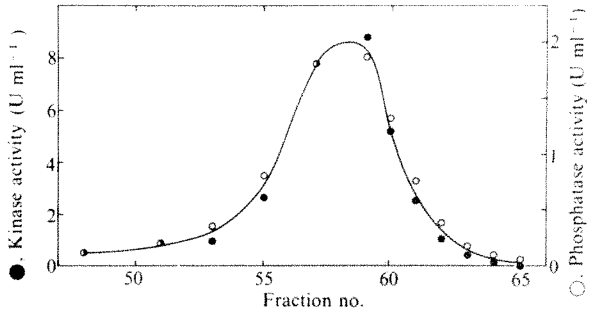

Fig. 2 Chromatography of IDH kinase and IDH phosphatase on DEAE–Sephacel. Following cellular disruption, centrifugation and ammonium sulphate precipitation, the sample was dialysed against buffer B (25 mM MOPS *p*H 7.0, 100 mM NaCl, 1 mM EDTA, 2 mM 2-mercaptoethanol), then applied to a DEAE–Sephacel column (2.5 × 35 cm) equilibrated in buffer B. After application, the column was washed with 100 ml buffer B and eluted with a linear gradient from 100 to 300 mM NaCl (750 ml each). Fraction size was 10 ml, flow rate 150 ml h^{-1}. IDH kinase was assayed by its ability to inhibit IDH in an ATP-dependent manner. The reaction mixture contained buffer C (25 mM MOPS *p*H 7.5, 5 mM $MgCl_2$, 100 mM NaCl, 2 mM dithiothreitol, 1 mM EDTA), 1 mM ATP, 0.5 mg ml^{-1} BSA and 0.6 μM IDH in a final volume of 50 μl. The reaction was started by adding ATP and terminated by addition of 50 μl 20 mM EDTA. Aliquots were then withdrawn and assayed for IDH activity in a reaction mixture containing 25 mM MOPS *p*H 7.5, 250 μM DL-isocitrate, 100 μM NADP, 5 mM NaCl. One unit of IDH kinase will produce 50% inhibition of IDH in 1 min. Calculations consider the IDH kinase reaction to be pseudo-first order with respect to IDH. IDH-phosphatase was assayed by its ability to catalyse the release of ^{32}P from phospho-IDH. The reaction mixture contained buffer C, 1 mM ATP, 2 mg ml^{-1} BSA and 0.1 μM ^{32}P-phospho-IDH (~20,000 c.p.m.) in 100 μl. The reaction was begun by addition of phospho-IDH and stopped by adding 150 μl of 20% trichloroacetic acid. The precipitated protein was removed by centrifugation and aliquots of the supernatant withdrawn for determination of ^{32}P. One unit of IDH phosphatase will catalyse the release of 50% of the ^{32}P in 1 min. Calculations consider the IDH phosphatase reaction to be pseudo-first order with respect to phospho-IDH. Typically, less than 20% of the phospho-IDH was hydrolysed during the reaction.

Initial studies of the IDH phosphatase showed that the activity in crude extracts was too low to account for observed rates of dephosphorylation *in vivo* and was very unstable as regards purification attempts. In resolving these problems, we discovered that the phosphatase activity had an absolute requirement for ATP or ADP. The activator has actually been shown to be Mg-ATP and all studies have been performed in 5 mM Mg^{2+}. This observation, together with the co-purification of kinase and phosphate activities, suggested that the activity identified as IDH phosphatase was, conceivably, the back reaction of the kinase. In such a case, ADP should be a far superior substrate as the ATP-dependent activity would actually result from the presence of ADP, either as a contaminant of the ATP or generated from it during the assay. In fact, ATP was found to be the superior substrate, with Michaelis–Menten constants of 100 and 400 μM for ATP and ADP, respectively.

To add weight to the conclusion that the ATP-dependent release of phosphate from phospho-IDH did not result from the ADP-dependent back reaction of the kinase, we tested the effect of adding an ATP regenerating system (Table 1). As such a system efficiently converts ADP to ATP, it would be highly inhibitory to any ADP-dependent reactions. The ATP regenerating system had no effect on the ATP-dependent phosphatase activity. When the ADP-dependent activity was examined, conversion of ADP to ATP by the regenerating system actually increased the phosphatase activity to a level slightly greater than that observed with an equivalent concentration of ATP; this increase appears to be due to an unidentified minor contaminant in the ADP preparation.

The products of the kinase back reaction are ATP and IDH. Thus, if the kinase back reaction were responsible for the release of ^{32}P from ^{32}P-phospho-IDH, the label should appear in ATP. When the products of the ATP- or ADP-dependent phosphatase reactions were treated with activated charcoal, we found that none of the released ^{32}P was absorbed, although the nucleotide was absorbed quantitatively. This observation, taken together with the results given above, indicates that the ATP-dependent phosphatase reaction is not the back reaction of the kinase.

The location of IDH kinase and phosphatase on the same protein resembles two previously described systems. It has been shown that the enzymes responsible for the adenylation–deadenylation of glutamine synthase in *E. coli* are located on the same polypeptide chain[8,9]. In addition, it has been reported recently that the rat liver 6-phosphofructo-2-kinase and fructose-2,6-bisphosphatase activities, which catalyse synthesis and breakdown of fructose-2,6-bisphosphate, are also physically associated[10,11]. It is interesting to note that, in addition to representing examples of the physical association of opposing activities, all three enzyme systems have major roles in the regulation of intermediary metabolism.

The possible advantages of localizing opposing activities on the same protein presents an intriguing problem. The occurrence of at least three such systems suggests that this is not an evolutionary accident. Such a physical association might have the advantage of allowing both activities to be controlled from a single site on the protein. While such a simultaneous regulation may occur, this is not the only mechanism by which such effects can be achieved. Simultaneous regulation could occur as a result of the interaction of a ligand with two distinct sites, one associated with each activity. Alternatively, in cases where the interconverted molecule is a protein, effectors which bind to that protein could result in simultaneous regulation. Nevertheless, physical association of opposing activities might represent a favourable mechanism for simultaneous regulation.

In addition to the potential for simultaneous regulation, the physical association of kinase–phosphatase for IDH may have been important in the evolution of the protein. The phosphatase site may have arisen from the kinase site by an imperfect gene duplication, possibly including a fusion event. If the imperfect duplication of the kinase site produced a phosphatase activity, the new site would be already designed to act on the common substrate. Although speculative, this suggestion explains the ATP requirement with the nucleotide achieving the same induced conformational change as for the kinase reaction, but with the specificity of the acceptor function altered to allow access by water.

Table 1 Effect of an ATP regenerating system on IDH phosphatase activity

Nucleotide		Phosphatase activity (mU)
ATP (0.1 mM)	No addition	12.4
	+ATP regenerating system	11.9
ADP (0.1 mM)	No addition	4.4
	+ATP regenerating system	14.0

Phosphatase assays were performed as described in Fig. 2 legend. Activity is presented in milliunits (mU). The ATP regenerating system consisted of 2 mM phosphocreatine and 10 U ml^{-1} creatine phosphokinase.

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A second plasma calcium-lowering peptide from the human calcitonin precursor

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Calcitonin is a 3,500-molecular weight (MW) polypeptide whose main function in man is to protect the skeleton during growth, pregnancy and lactation[1]. It is secreted by C-cells which are localized in the thyroid in mammals, but may be present mainly in the ultimobranchial body and lung of more primitive vertebrates[2]. The hormone is also concentrated in the hypothalamus of several vertebrate species[3,4], including man[5], and in the primitive brain of the chordate *Ciona intestinalis*[6,7]. Based on cloning and subsequent analysis of recombinant DNA, recently we have reported the partial nucleotide sequence of the human calcitonin precursor mRNA[8,9]. This study showed that calcitonin resides towards the C-terminal end of its precursor protein, flanked by cryptic peptides of unknown significance. We now report that the synthetic C-terminal flanking peptide (PDN-21) predicted from the nucleotide sequence is highly active biologically—it approaches the potency of calcitonin in reducing the level of plasma calcium. As immunological evidence suggests that PDN-21 is synthesized *in vivo* and circulates in man, it may represent a novel hormonal cleavage product from the human calcitonin precursor with a physiological role in Ca^{2+} regulation.

Increasing numbers of small peptide hormones have been shown to be synthesized as high molecular weight precursor proteins that are subsequently processed to yield one or more peptides of biological significance[10,11]. Thus it was of interest to determine whether the predicted peptides flanking human calcitonin within the precursor possessed biological activity or were present only to ensure correct processing of the calcitonin precursor protein[9].

To investigate this possibility, the human C-terminal flanking cryptic peptide (H-Asp-Met-Ser-Ser-Asp-Leu-Glu-Arg-Asp-His-Arg-Pro-His-Val-Ser-Met-Pro-Gln-Asn-Ala-Asn-OH) corresponding to the last 21 amino acid residues predicted from the coding sequence of the calcitonin precursor mRNA[9], was

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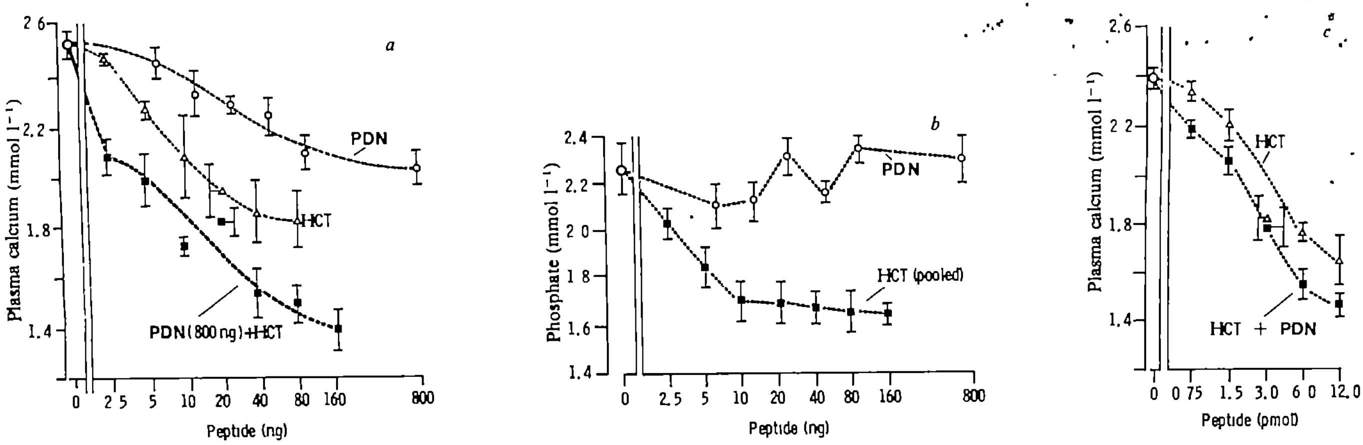

Fig. 1 Effect of PDN-21 on plasma calcium and phosphate. After preliminary experiments had shown that the time courses of the effects of PDN-21 and human calcitonin (HCT) on plasma calcium were similar, peptide was injected in sodium acetate buffer (pH 4.6) into the tail vein of female Wistar rats (mean weight 45 g, range 42–48 g; Oxfordshire Laboratory Animal Colonies) and blood taken from the aorta after 30 min in a randomized bioassay[23]. *a*, Plasma calcium was measured colorimetrically by the method of Gindler and King[24] and the values confirmed by atomic absorption spectrophotometry. Each point is the mean ± s.e.m. from three rats. ○, PDN-21; △, human calcitonin (WHO 1st International reference preparation of calcitonin hormone for bioassay 70/234, supplied by the National Institute for Biological Standards and Control); ■, human calcitonin + PDN-21 (800 ng PDN-21 was injected with each dose of calcitonin). Analysis of variance showed highly significant ($P < 0.001$) dose-related plasma calcium differences among the three treatment groups. The dose giving 50% response (ED_{50}) was 7 and 24 ng for human calcitonin and PDN-21, respectively. Over the linear dose–response range, 1 ng of PDN-21 had the same effect as 0.17 ng human calcitonin; in the human calcitonin + PDN-21 group, 1 ng human calcitonin was as active as 5 ng given alone. Three previous unrandomized experiments gave essentially similar results. *b*, Plasma inorganic phosphate[25,26] was measured in the same rats shown in *a*. PDN-21 produced no significant change at any dose level (○) but both the calcitonin and calcitonin + PDN-21 groups showed highly significant ($P < 0.001$, analysis of variance) dose-related reductions. These latter two groups did not differ and the results were therefore pooled (■). Values shown are mean ± s.e.m. *c*, In a separate batch of female Wistar rats (mean weight 51 g, range 51–53 g) from the same supplier, the effect of calcitonin alone (2.5–40 ng; △) was compared with that of calcitonin injected with equimolar amounts of PDN-21 (1.8–28 ng; ■). Each point is the mean for five rats ± s.e.m. The ED_{50} was 15 and 9 ng for human calcitonin alone and for calcitonin plus PDN-21, respectively. When equimolar PDN-21 was present, 1 ng of calcitonin was as active as 1.6 ng calcitonin alone (95% confidence limits 1.2–2.1 ng).

synthesized (Peninsula Laboratories) by solid-phase methodology, and the identity and homogeneity of the product established by amino acid analysis, high voltage electrophoresis and reverse-phase HPLC. We have called the peptide PDN-21 according to the terminology of Tatemoto and Mutt[12]. This peptide was used to obtain preliminary evidence of biological activity and to raise the antisera required to establish its presence in human tissues and sera.

Initial pharmacological studies yielded striking results. PDN-21 is highly active in reducing plasma Ca^{2+} in the rat (Fig. 1*a*) although the maximum effect is less than that produced by calcitonin. Our findings also indicate that the potency of human calcitonin is increased by the presence of PDN-21; the enhancement was fivefold (Fig. 1*a*) when maximal doses were used but still 60% when equimolar PDN-21 was given with human calcitonin (Fig. 1*c*). As the two peptides are presumably secreted in equimolar amounts, these results imply that PDN-21 may be important in the regulation of plasma Ca^{2+} *in vivo*. Further, because the effect of PDN-21 on calcium is additive to that of calcitonin, it is possible that the peptide acts at a different receptor. This action of PDN-21 (800 ng) is still obvious in nephrectomized animals (Fig. 2) and is therefore unlikely to be due to loss of Ca^{2+} but rather to a redistribution, perhaps involving bone. This is consistent with results from 48-h mouse calvariae explants in culture (Table 1); there is clear evidence of a direct effect of PDN-21 on ^{45}Ca-release from the prelabelled bones ($P = 0.02$ at the higher dose). As yet, we have not determined whether PDN-21 has an important role in Ca^{2+} regulation and bone physiology. It is also intriguing to speculate whether the hypocalcaemia that follows hypercalcaemic thyroid perfusion[13] is due solely to the effect of calcitonin release, or depends also on this new candidate hormone.

It is important to note that this effect on plasma calcium is highly specific. Thus, no effect was observed on plasma sodium, potassium, albumin or plasma phosphate (Fig. 1*b*). This latter finding clearly distinguishes PDN-21 from calcitonin, which lowers both plasma calcium and phosphate[14]. The glucose and magnesium levels did not differ significantly in relation to dose although the latter were slightly higher overall. There were no effects in guinea pigs and rats on smooth muscle contraction (ileum and bronchus), urine flow, milk ejection pressure or blood pressure.

Our experiments thus establish the biological activity of PDN-21 and suggest it has a role as a calcium-lowering hormone in man. However, immunoassay with antiserum specific for

Table 1 Comparison of the effectiveness of human calcitonin and PDN-21 in inhibiting cell-mediated ^{45}Ca-release from prelabelled mouse calvariae *in vitro*

	% Inhibition of cell-mediated ^{45}Ca-release ± s.e.m.
PDN-21	
40 μg ml^{-1}	63.6 ± 14.3 (5)
10 μg ml^{-1}	25.5 ± 9.9 (4)
Human calcitonin	
0.2 μg ml^{-1}	62.8 ± 8.2 (5)
0.02 μg ml^{-1}	48.6 ± 10.5 (7)

The method for studying the response of mouse bones to calcitonin preparations has been described in detail[21]. Cell-mediated release of ^{45}Ca into the culture medium was calculated by substracting the amount of exchangeable ^{45}Ca released into the medium by dead explants of 6-day-old prelabelled mice of the same litter from that released by the living treated explant[22]. The number of treated explants is shown in parentheses; the data were obtained after treatment of explants for 48 h *in vitro*. Although a higher dose of PDN-21 than human calcitonin was required, no attempt has been made to calculate 'potency' ratios because the dose–response curves for human calcitonin and PDN-21 are not parallel, and the relative stability of the peptides in the 48-h culture conditions is unknown. More work is necessary to establish the nature of the action of PDN-21 on explants and whether it is a true inhibition of resorption. Similar experiments with explants prelabelled with ^{3}H-proline, which is incorporated into collagen, should be rewarding. In tests on uptake of ^{45}Ca into explants *in vitro* there did not seem to be any effect of PDN-21 over controls, therefore we conclude that it probably does not alter bone accretion.

PDN-21 shows that the peptide circulates in concentrations approximately equimolar with calcitonin in human medullary carcinoma of the thyroid (Fig. 3). As PDN-21 is also measurable in normal human thyroid tissue, the peptide presumably circulates in a healthy subject, suggesting a normal hormonal role. Although studies of the action of the equivalent rat calcitonin C-terminal peptide (CCP)[15,16] have not been reported, measurements in the rat are in agreement with our results from immunoassay. Thus, antisera against synthetic CCP show that this peptide is present in equimolar amounts with calcitonin in normal rat thyroid tissue, rat medullary carcinoma of the thyroid (MCT), and is secreted by monolayer cultures of rat MCT cells[17,18].

Since the submission of this work, Amara and co-workers[19] have described alternative, apparently tissue-specific RNA processing of rat calcitonin gene transcripts, resulting in the generation of mRNAs encoding two different polypeptide products: calcitonin, and a calcitonin gene-related peptide (CGRP). Calcitonin mRNA was the major species in the normal thyroid but CGRP mRNA predominated in the hypothalamus. The equivalent splicing mechanism giving rise to a human CGRP-like mRNA has yet to be described and it remains to be determined whether or not rat CGRP mRNA is translated into an active product in the brain.

Two main conclusions follow from our work. First, the human calcitonin precursor is a true polyprotein with at least two biologically active cleavage products and falls into the group of precursor proteins typified by pro-opiomelanocortin. Second, in addition to a possible involvement in Ca^{2+} regulation in health, PDN-21 may have therapeutic potential. Its intrinsic activity on calcium, together with its enhancement of the activity of calcitonin, suggests that it may be useful in the treatment of hypercalcaemia or of Paget's disease, where human calcitonin is at present the treatment of choice[20].

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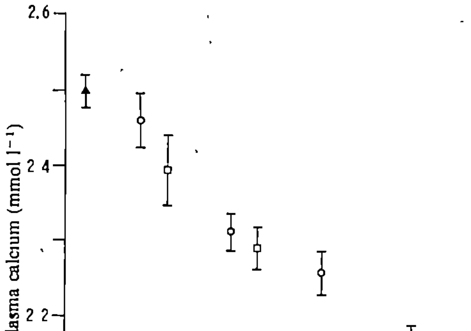

Fig. 2 The effect of nephrectomy on the calcium-lowering action of PDN-21 and calcitonin. Female Wistar rats (mean weight 56 g, range 57–58 g; Charles River, UK) were divided into groups of six, anaesthetized with ether and the kidneys exposed from a posterior incision (sham groups, □) or removed (○). Acetate buffer alone (0.2 ml), PDN-21 (800 ng), human calcitonin (40 ng) and both peptides together, were injected in buffer immediately after operation into both sham and nephrectomized groups. All the peptide preparations produced highly significant calcium reductions ($P<0.005$). The action of PDN-21 was unaltered by nephrectomy although human calcitonin had a slightly smaller effect after removal of the kidneys ($0.01<P<0.05$). The control group (▲) was injected with acetate but not anaesthetized Rats from this supplier are slightly less sensitive to both human calcitonin and PDN-21.

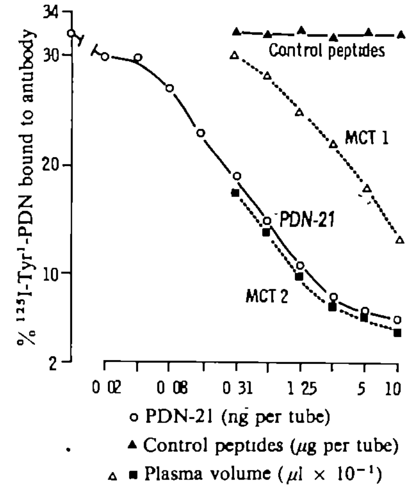

Fig. 3 Immunoassay of PDN-21 in human plasma. Tyr^1-PDN-21 (Peninsula Laboratories) was coupled to ovalbumin[27] and an antiserum raised in rabbits. The serum was used at a dilution of 1:1,200 with ^{125}I-Tyr^1-PDN-21 as tracer. The presence of 10 μg per tube of luteinizing hormone-releasing hormone, salmon calcitonin, human calcitonin, somatostatin, ACTH$_{1-24}$ (Synacthen; Ciba-Geigy), porcine ACTH$_{1-39}$, human parathyroid hormone, oxytocin, insulin, pro-opiomelanocortin, human prolactin, or human growth hormone did not interfere in the assay (▲). The limit of sensitivity was ~40 pg per tube, the intra-assay variability <5% and the inter-assay variation ±10%. We studied plasma from 16 patients with MCT proven at operation; parallel displacement curves were found in 15 of the 16 cases Plasma PDN-21 ranged from 0.6 to 138 ng ml^{-1} and was approximately equimolar with human calcitonin (range 0.8–314 ng ml^{-1}). Results for plasma dilutions from two of the 16 MCT patients are shown together with a displacement curve for synthetic PDN-21 (○). Patient 1 (MCT 1, △): PDN-21 11 ng ml^{-1}, human calcitonin 18 ng ml^{-1}; patient 2 (MCT 2, ■): PDN-21 103 ng ml^{-1}, human calcitonin 314 ng ml^{-1}. As expected due to its equimolar relationship to normal calcitonin levels of 20–100 pg ml^{-1}, PDN-21 was not measurable in 12 normal subjects at this assay sensitivity.

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BOOK REVIEWS

Men of science, men of letters

L. Pearce Williams

NATHAN and Ida Reingold, historian of science and mathematician respectively, have conceived the bold idea of presenting the history of science in America from the beginning of the twentieth century until the outbreak of the Second World War in Europe in terms of letters written by American men of science. The various sections devoted to the organization of science in America and the development of the various sciences are prefaced by short essays by the editors intended to introduce the *dramatis personae* and to sketch in the background against which the letters may be intelligently viewed and considered. The work, as a whole, is introduced by a short section whose main purpose is to define science in its American context and to indicate the place that science played in twentieth century America.

There is a notable virtue to this approach, and I should like to stress it at the outset of a review which will be generally critical. The reader is offered a splendid feast of hitherto unpublished and utterly fascinating primary sources. There are letters from almost all the "greats" of modern American science, containing important insights into their personalities, their concern for the public face of science and their own scientific work. Here are candid remarks on the organization and content of American science from William Henry Welch, Ira Remsen, George Ellery Hale, Charles W. Eliot, Irving Langmuir, Norbert Wiener, Jacques Loeb, Alfred H. Sturtevant, Albert Einstein and John von Neumann, to mention only the most famous, with replies from such non-American scientists as Ernest Rutherford, Wilhelm Ostwald and Bertrand Russell. As these names indicate, the sciences of astronomy, medicine, genetics and physics are well represented and readers will be able to discern what the major problems, both social and intellectual, were which concerned these men in the twentieth century. By publishing these letters, the Reingolds have done an invaluable service to historians of American science who might not have recognized the riches that were at their disposal, and by calling attention to the depositories in which these letters were found they have indicated rich veins for further exploration.

In this sense, this is an indispensable volume for all students of the modern American scene and I recommend it highly to them. It should also be on the library shelf of every practising scientist over the age of 40 who will find in it echoes of his own education and scientific upbringing. Such persons will read it with an enjoyment that the non-scientist cannot share. This is, then, an important work but it is also deeply flawed and it is to its flaws that I must now turn.

Science in America: A Documentary History 1900–1939. Edited by Nathan Reingold and Ida H. Reingold. Pp.490. ISBN 0-226-70946-9. (University of Chicago Press: 1982.) $49, £26.25.

In their brief preface, the Reingolds wrestle with a problem that has bedevilled historians of science now for a generation, namely the "internal" versus "external" perspectives on the history of science. The first states that science is an autonomous activity that develops by an internal logic of its own; the second insists that scientists reflect the general culture that surrounds them in their own scientific works. Non-historians of science may well be puzzled that serious scholars find much to argue about here since common sense indicates that both propositions are true. There *is* an internal logic to the sciences and scientists *do* reflect their society. The trick is to discover when each of these applies.

The Reingolds are perfectly aware of the fact that science has both a public and a private face, and they try to deal with this by means of a metaphor. Instead of speaking of a "core" of hard science, dear to the internalist, and an "outside" to which the externalist appeals, they assume that

> there is no core — everything is part of an interface. And if the core with its weighty contents, has the aura of permanence, the interface is like a thin film, almost a soap bubble. Ideas, people, institutions of all sorts — not merely the scientific — exist in the interface. It is too much for such fragility, and tensions are created which produce additional film.

I simply do not understand what this metaphor means. How science exists in a soap bubble, or how tensions at the interface create more film escape me. Nor does the metaphor do much for the Reingolds except to permit them to insist that pure and applied science are both important, and that scientific institutions as well as private biases and prejudices go into the making of science. No one, again, will argue with this conclusion which could have been presented without the metaphor.

The metaphor, however, does get the Reingolds into serious trouble. By defining science as part of an interface, they eliminate any distinction between the private and public face of science. This, then, permits them to publish a book containing documents drawn solely from the private sector and clearly intended by them to be a documentary history of science in America. I have often criticized historians of science whose works have included no references to private papers because they seemed to me to be seriously deficient. This work sins equally heavily on the opposite side. We read a number of letters by George Ellery Hale on various topics, but no reader of this volume would be able to describe Hale's contributions to modern astronomy in any detail. Similarly, the great physiologist, Jacques Loeb, is well represented by letters that, occasionally, refer to his epoch-making scientific work but not a single one of his important published articles is present.

The list of such omissions is as long as the list of letters and any reader who expects to gain an understanding of the course of scientific thought in America in the first third of the twentieth century may be bitterly disappointed by this book. Had it been entitled, *American Scientists: A Documentary History*, there would have been a much closer relationship between title and contents.

Having already praised the variety and interest of the letters printed by the Reingolds, I should also raise some questions about their editorial judgement in presenting them. The editors try to disarm critics by announcing that "the documents are edited with a decent minimum of editing". I should like to disagree. Important information is withheld and this vitiates some of the scholarship involved. One of the fundamental commandments of the editing of historical documents is to locate such documents in both time and place. Throughout the volume, place is systematically excluded. This leads, at best, to confusion as the reader tries to work out where the writer is so that he can understand local allusions. At worst, it makes parts of the letters unintelligible. Works such as this should not force their readers to guess where information is available. This is not a trivial point — anyone wishing to use these letters in other works of scholarship will have to go to their sources to find out this information, thus forcing a duplication of effort that could easily have been avoided.

While I agree that footnotes to letters should be as short and concise as possible, it is important that they also provide the information necessary for the reader to understand what he is reading. In too many letters, there are names which are not identified. When Alfred Sturtevant urges Milislav Demerec "to try and get hold of Pink Jesus (= Bug Forbes)" (p.327), I suspect that few readers will understand the allusion. Professor Forbes was one of Cornell's legendary figures whose beard and ruddy complexion gave him his nickname and who certainly deserves a footnote. In other cases, a person is identified, if giving a first name, last name and dates of birth and death can be counted as identification — surely a brief description of a person's place in science would have been helpful? The Reingolds also do not always provide essential information for their readers about scientific matters. In the same letter from Sturtevant and the one immediately preceding it, Sturtevant mentions *Oenothera lamarckiana.* How many readers will immediately recognize this as the plant used by Hugo de Vries in his studies of mutation? The Reingolds say nothing.

Although these are serious criticisms, I would not like to close on a sour note. This is not an adequate documentary history of science in America, as its title insists; but it is a fascinating slice of scientific Americana that should prove both stimulating and useful to a wide variety of readers. □

L. Pearce Williams is the John Stambaugh Professor of the History of Science at Cornell University. He is the author, among other works, of Michael Faraday, A Biography *(Chapman & Hall, 1965).*

In terms of analytical chemistry

T.S. West

A Dictionary of Chromatography, 2nd Edn. By R.C. Denney. Pp.229. UK ISBN 0-333-31667-3; US ISBN 0-471-87477-9. (Macmillan Press/Wiley: 1982.) £15, $39.95. *A Dictionary of Spectroscopy,* 2nd Edn. By R.C. Denney. Pp.205. UK ISBN 0-333-31670-3; US ISBN 0-471-87478-7. (Macmillan Press/Wiley: 1982.) £15, $39.95.

THESE two second-edition dictionaries of chromatography and spectroscopy are attractive additions to the chemist's bookshelf, where they should serve as useful guides to those not fully expert in either technique. The author has successfully put together a reasonably comprehensive set of terms and definitions and has translated them into SI terminology in keeping with present-day trends. The terminology of the IUPAC "Orange Book" of Analytical Nomenclature has been adhered to fairly closely, and the author has steered a consistently sagacious path through the minefield of inconsistencies that abound in these as in most other areas of nomenclature.

I was, however, disappointed to find no mention of "limit of detection" in either book or "sensitivity" in the spectroscopy volume. Both volumes similarly define the term "signal-to-noise", but in neither is it said exactly what a signal is and the explanation of noise will not be very helpful to the puzzled novitiate, even in the chromatography volume. However, terms such as these are perhaps the most difficult to define and the author may have been wise to avoid or define them minimally. On the other hand, useful reference could certainly have been made to other terms such as "time-resolved spectra" and "background", particularly for the newcomer to these techniques.

In both books the author has made excellent use of explanatory diagrams, 55 each for both chromatography and spectroscopy, and in general the definitions are clear and very much to the point, particularly those in the chromatography volume. Each book includes an extensive list of references for further reading, but a glossary of the terms defined, with the page numbers, would have been useful even though the two books are alphabetical lists from start to finish.

The chromatography volume gives the impression of being more comprehensive and perhaps more sure-footed than that on spectroscopy. However it may well be that the latter serves a more useful purpose in that it deals with some of the newer electron spectroscopies — such as Mössbauer and Auger spectroscopy — that are less well covered in other dictionaries which tend to concentrate exclusively on the more familiar molecular, atomic and electron spectroscopy techniques.

Each book is timely and excellent in conception, and in remarking that I would like to have seen such and such a term defined, I am basically expressing my satisfaction with the other definitions and wishing that there could have been more of the same. Both volumes deserve to sell well; they are especially recommended for student use. □

T.S. West is Director of the Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen.

Integrated enzymes

Athel Cornish-Bowden

Fundamentals of Enzymology. By N.C. Price and L. Stevens. Pp.454. Hbk ISBN 0-19-857175-5; pbk ISBN 0-19-857176-3. (Oxford University Press: 1982.) Hbk £25, $49; pbk £12.50, $24.95.

ENZYMOLOGY has a role in nearly all aspects of research in biochemistry, and is of central importance in the whole subject. Partly because of this, it is commonly taught to undergraduate students as a series of disconnected topics. The people who study protein structure often have little in common with those concerned with the control of metabolic processes in the living organism. A third group of people study enzyme kinetics, and among these there sometimes seems to be an unbridgeable gulf between those who are interested in the steady state and those who are interested in transients. Yet these and other aspects of enzymology are all part of the same subject and it is clearly desirable to integrate them.

The present book is an attempt to describe enzymology as a whole, from the structures and properties of isolated enzymes to their roles as components of complex metabolic systems. It is written at a level where it can easily be understood by undergraduates, but it has enough detail to be of value to any research worker with an interest in enzymes. It is not the first book to attempt such a synthesis, but it is easily the most successful that I have seen, and it deserves to become a standard textbook of general enzymology.

The book is informative and accurate about the aspects of the subject that I know best, and so I feel inclined to believe what it tells me when it ventures into less well known territory, for example (in a final chapter on enzyme technology) about the origin of the holes in Swiss cheese. I found few faults, none of them serious. Although the authors are not themselves confused about the distinction between the Hill coefficient and the number of binding sites on a protein, they may well confuse their readers because they use the same symbol for both quantities on nearly consecutive pages.

Although monstrosities such as SGOT make fleeting appearances (inevitable, perhaps, in a chapter on clinical enzymology), and molecular weights are mentioned throughout, the authors have in general made commendable efforts to refer to enzymes by their recommended names and to use modern units and terminology. Even the clinical chapter is largely written in English, perhaps the first time this has ever been achieved. □

Athel Cornish-Bowden is a Lecturer in Biochemistry at the University of Birmingham, UK, and author of Basic Mathematics for Biochemists *(Chapman & Hall/Methuen, 1981).*

Scattering information from Graz

Henryk Eisenberg

Small Angle X-ray Scattering. Edited by O. Glatter and O. Kratky. Pp.515. ISBN 0-12-286280-5. (Academic: 1982.) £43.60, $81.

FOR SOME years study of the scattering of radiation has been a favoured approach in the investigation of a variety of low molar mass, colloid and biological systems. There are three principal techniques: light scattering which, using an effective wavelength of a few thousand Angstroms, has been revolutionized by the advent of the laser; small angle X-ray scattering, which is restricted to low scattering angles because of the much lower wavelength (around one Angstrom); and neutron scattering, which has recently come to the fore and shows greater flexibility with respect to wavelength (from around one to a few tens of Angstroms). Although the three phenomena share the same basic theory, interesting complementarities result from the specific nature of each of the processes.

Recently, small angle X-ray scattering has received a strong impetus following the introduction of one- and two-dimensional position-sensitive detectors, computerized data acquisition and treatment, and the use of powerful synchrotron radiation sources. These developments have shortened experimental times to the subsecond range, allowing the study of dynamic phenomena. Long viewed with suspicion because of unwieldy and error-prone procedures, and a tendency towards overinterpretation of an essentially information-poor experimental technique, small angle X-ray scattering has now matured into a position of considerably higher respectability.

Although the editors have evidently made efforts to broaden the spectrum of contributors, the book under review represents to a large extent the individual and collective achievements of the Graz school of Kratky and Porod. Porod himself presents a comprehensive and lucid discussion of the general theory, which owes much to his efforts. Statistical fluctuations are mentioned, though not in sufficient depth to relate them to the analysis of multicomponent systems. Instrumental methods, and data treatment and interpretation are covered in detail in the various chapters, as well as contrast variation, a trade-mark procedure in neutron scattering. Although the underlying theory is identical for X-rays and neutrons, no equivalent exists to the contrast variation achieved by manipulation of hydrogen–deuterium compositions in the neutron technique.

Discussions of applications range widely over proteins, nucleic acids and nucleoproteins, lipoproteins and membranes, and natural and synthetic high polymers in the dissolved and solid state, including evaluation of the Kratky–Porod persistent chain, inorganic substances, and aggregates and micellar structure of small molecules in solution.

This volume is a useful though somewhat uneven compilation. Thus, in parts, quantities in equations are not properly defined, some awkward expressions intrude, and references are missing or incomplete. Also it is irritating that different symbols are sometimes used for the same parameter, even in the same chapter.

Altogether, however, the book does provide a good source of information for evaluating both past achievements and future promise in an important field of study. For those wishing to come to grips with most of the theoretical and practical aspects involved it will prove instructive reading. □

Henryk Eisenberg is a Professor in the Polymer Department at the Weizmann Institute, Rehovot, Israel.

The right time for retroviruses

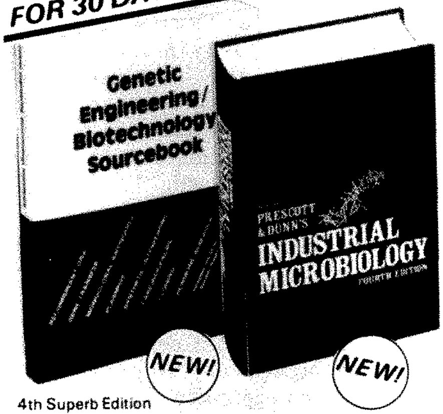
Neil Wilkie

RNA Tumor Viruses. Molecular Biology of Tumor Viruses, Part 3, 2nd Edn. Edited by Robin A. Weiss *et al.* Pp.1,408. ISBN 0-87969-132-8. (Cold Spring Harbor Laboratory: 1982.) $125 (US), $150 (elsewhere).

ALL YOU ever wanted to know (and even more) about retroviruses, contained in one volume. That seems to be the promise of the new Cold Spring Harbor publication *RNA Tumor Viruses,* which together with the companion volume on DNA tumour viruses comprises the second edition of the popular series *Molecular Biology of Tumor Viruses.*

By and large, that promise is fulfilled by a notably clear and up-to-date review of a complex and fast-moving field. The decade elapsed since the publication of the first edition has witnessed remarkable progress in our understanding of the structure and function of retroviruses. It is not surprising, then, that the current volume is massive (it weighs 2 kg and runs to 1,400 pages) or that is essentially a completely new book, rather than a revision of the first edition.

The stated aim of the book is to cater for both the novice and the expert, but novice and expert in what field? The study of



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retroviruses has provided insight into many important areas of biology removed from "straight" virology. Retrovirus genomes can be inherited as Mendelian genes as well as spread between and within species by infection; they mimic the effects of mobile genetic elements by mutation and alteration of the expression of chromosomal genes; they activate, recombine with and transduce cellular oncogenes; and they induce defects in the regulation of cell growth. The book is therefore of direct interest to virologists, molecular biologists, geneticists, cell biologists, tumour biologists and clinicians.

Does the book work? The text is divided into 11 chapters which deal firstly with molecular aspects of the genome, its replication and expression, and then with the biological aspects of expression, infection and pathogenesis. The self-contained chapter structure has resulted in considerable repetition and contributes to the overall length of the book. This is anticipated in the preface and on balance the strategy is acceptable; a little reiteration does no harm when the subject matter is so complex. The text has been researched in such formidable detail that complete novices may find some sections hard going. I imagine that undergraduates, for example, would need some guidance in the correct usage of the book. Nonetheless, the writing is generally clear and in most chapters there should be no difficulty in abstracting the information at different levels of complexity. Moreover, where doubt or ambiguity of interpretation is present, this is usually pointed out and the alternatives explained.

I particularly enjoyed the three chapters on the molecular biology of the retrovirus genome. They clearly explain the exquisite mechanisms by which the RNA genome is converted to a DNA copy in which a terminally redundant LTR sequence is created, how this is inserted into the chromosome and expressed using tactical variations on a strategy evolved to permit the synthesis of many gene products from a single genome sequence. The sections covering endogenous viruses also successfully deal with a complex subject while highlighting the evolutionary implications. Of course the pathogenicity, particularly the oncogenicity, of retroviruses is currently a centre of attention and readers will find the chapters on pathogenicity and oncogenes to be of especial interest. These subjects are, in the main, well treated. Just enough (and no more) information on haematopoiesis is given to understand the concepts involved in virus-induced cellular disease. The several current models for the mechanistic interpretation of transformation are fairly represented and the review of oncogenes and their products is also well-balanced and up-to-date.

The final chapter on the identification of human RNA tumour viruses explains the dangers of overinterpreting initial results and introduces the only convincing isolate to date: the lymphoma-leukaemia virus associated with certain forms of adult T-cell leukaemia. A sting in the tail of the book is provided by a compilation of restriction endonuclease maps and primary nucleotide and amino acid sequences of some well-studied viruses.

Students of retrovirology and its practitioners will be not unaware of the clashes which often arise between some dominant personalities with individual and exclusive viewpoints. Although only a small number of authors could be included in the writing of the current volume, my impression is that this has not been allowed to disturb the balance of the text and that the views expressed are reasonably representative of the field (not always the case in other volumes from the same stable).

I also sense that the timing of the book is critical: we are probably ending a phase in which details of the virus structure are the main concern, and beginning one in which the consequences of virus–cell interactions will predominate. This volume comfortably spans these areas. It is exhaustively researched and referenced, and will provide the standard reference text for the field for the foreseeable future.

Neil Wilkie is Head of the Tumour Virus Laboratory at the Beatson Institute for Cancer Research, Glasgow.

An English Lamarck

An English translation of Madeleine Barthélemy-Madaule's *Lamarck ou le Mythe du Précurseur* (Editions du Seuil, 1979) has just been published by MIT Press. The original edition was reviewed in *Nature* **286**, 187; 1980.

The translation (by M.H. Shank) has the title *Lamarck the Mythical Precursor*, and costs $17.50, £12.25.

Looking at comets in Arizona

David W. Hughes

Comets. Edited by Laurel L. Wilkening. Pp.766. ISBN 0-8165-0769-4. (University of Arizona Press: 1982.) $29.95.

TUCSON, Arizona was the place and March 1981 the time of the meeting of 175 scientists for the Sixty-first International Astronomical Union's Colloquium on "Comets: Gases, Ices, Grains and Plasma". The review papers presented at that meeting provide the basis for this book.

Comets have been newsworthy for centuries and scientists are forever using the latest observational techniques and theoretical gambits to delve into their mysteries. The 1980s are no exception. The impending return of Halley's comet in 1986 should see some visits by spacecraft; the successful operation of the International Ultraviolet Explorer has made H and OH observations in the extended coma a commonplace occurrence; the collection of cometary dust in the Earth's upper atmosphere is now routine; and the Pioneer Venus orbiter observations of the interactions of the solar wind with an atmosphere unprotected by a magnetic field gave a preview of what is to be expected in the cometary case.

"New theories and data are collected in this book as an introduction to comets for newcomers as well as a reference volume for specialists" — I quote from Laurel Wilkening's preface. Unfortunately I don't think a book can easily be both and this one certainly is not. It contains 29 papers grouped under headings such as Overview; Nucleus; Dust; Coma, Ion Tails and Solar Wind Interaction; Origin, Evolution and Interrelations; and Basic Information and References. As with all conference proceedings the standard is mixed but there are some real gems — Delsemme on the chemical composition of cometary nuclei, Kresak on statistics and observational selection, Whipple on nuclei rotations, Sekanina on splitting, Wetherill and Revelle on the relationship between comets, meteorites and fireballs, A'Hearn on spectrophotometry, Feldman on the ultraviolet, Everhart on orbital evolution, Marsden and Roemer on astrometry and basic characteristics.

Such papers lift this conference proceedings from the category of "every library should purchase it" to "every comet researcher should have it on his personal bookshelf". If, however, critical comparisons must be made I do think the University of Arizona did a better job on the asteroids in 1979 than they have here on the comets. (For a review of *Asteroids*, edited by T. Gehrels, see *Nature* **287**, 664; 1980.)

Also, when it comes to introducing a topic to newcomers it must be ensured that certain features are not omitted. This is especially difficult for the editor of conference proceedings who is restricted not only by the ease with which scientists raise travel money, and their predilections for Arizona in March, but also by the problems of convincing the participants to write up their papers in a suitable form. There are far too many gaps in this volume to make it a good introduction for the newcomer. ☐

David W. Hughes is a Lecturer in Astronomy and Physics at the University of Sheffield.

nature

9 December 1982

Putting Europe's missiles at risk

Next year will be a bad year for European governments, perplexed by strategic problems and bothered by elections. The United States should help them out.

1983 promises to be a rowdy year for nuclear strategists, a testing time for the governments of states belonging to the North Atlantic Treaty Organization (NATO) and one of the most strenuous years for NATO itself since the row about the European Defence Force early in the 1950s and the departure of France at the end of that decade. Although the new year is still some weeks off, the pressure is already building up, principally about the decision taken unanimously by the NATO governments at the end of 1979 to deploy new intermediate range nuclear weapons in Western Europe towards the end of 1983. The plan is that 108 Pershing II ballistic missiles should be sited in West Germany, and that 362 ground-based cruise missiles should be based in West Germany, Italy, the Netherlands and the United Kingdom.

Predictably, the Soviet Union is doing everything it can to frighten governments in Western Europe with the consequences of their decision three years ago. Soviet spokesmen have taken to saying that the short flight-time of the Pershing missiles might force Soviet forces onto the hair-trigger strategy of "launch-on-warning", with all the dangers of accidents that would follow. There have also been heavy hints that it may be necessary to install more of the 5,000-kilometre SS-20 mobile missiles on the Soviet side, that sea-launched cruise missiles might be placed within range of the United States and even that ballistic missiles might again be based in Cuba. The Soviet Union's obvious objective is to undo the 1979 agreement. It is high time that Western governments worked out a resolute response.

The present, unfortunately, is an unpropitious time for working out a level-headed response. Of the principal governments involved in the 1979 decision, only the British government has not been changed, either by the ballot-box or by some other constitutional process. And while it might in ordinary circumstances have been expected that the new West German government of Chancellor Helmut Kohl would have been more able politically to carry through the 1979 agreement than its predecessor, haunted as it was by disagreements on nuclear policy within its own party, Chancellor Kohl is almost as much constrained by his promise to hold a general election next March. Elsewhere in Europe, even the strongest governments have been given pause by the resurgence of opinions dissenting from present strategies. The present British government, for example, has to contend with — and ideally must somehow answer — an increasingly vocal Campaign for Nuclear Disarmament that would abandon the plan to replace the British Polaris submarines with Tridents, decline to provide bases for US cruise missiles and eventually (but not yet) withdraw from NATO, a Labour opposition party now committed to the abandonment of Trident and even an opinion from a Church of England group tending in the same direction. What, in these circumstances, is to be done?

The first need is that embattled governments should remind their people of the reasons for the 1979 agreement and of its terms. The decision to base missiles of intermediate (as distinct from "tactical") range in Western Europe derives simply from the deployment of the Soviet force of SS-20s during the 1970s. With the help of the earlier SS-5 and SS-6 missiles, the Soviet Union could now deliver more than 1,000 warheads on targets in Western Europe using delivery systems that can have no wider strategic role. (The mobile SS-20s are also potentially a threat to China, but most of them just now are based in the central and western parts of the Soviet Union.) To be sure, both sides could carry larger numbers of nuclear warheads to targets some thousands of kilometres away by means of aircraft, but plainly there are technical advantages in a force of ballistic missiles and in the diversity that they bring. If some measure of strict military balance is a legitimate goal of strategic policy, the NATO decision of 1979 was almost unavoidable. On the view, for which there is a rich body of argument in justification, that the nuclear balance has helped to keep the peace in Europe in the past several decades, the decision may also be held to have been prudent.

What has been commonly overlooked, in the arguments between Western governments and their nuclear critics, is that NATO's decision in 1979 was deliberately conditional. The governments concerned said loudly and clearly that intermediate range missiles would be based in Western Europe by the end of 1983 only if, by then, there had been no substantial progress towards arms control in the European region. At the time, the West German government was chief among those that argued that no intermediate range missiles on either side would be preferable to other solutions.

The experience of the past several months at the Intermediate Nuclear Force negotiations (IFN) at Geneva is far from encouraging. So far, the United States has publicly proposed that all intermediate-range missiles should be dismantled, while the Soviet Union has countered in the private meetings with the suggestion that missile launchers should be limited to 300 on each side. Neither of these proposals can be expected to satisfy the other side, especially when account is taken of the complications arising from the substantial fleets of aircraft on each side, and the presence of independent strategic nuclear forces in both Britain and France.

The gap between the two views is not, however, unbridgeable. Technically, the urgent need is for some means of assigning potency to delivery systems of various kinds, and then for settling on appropriate upper limits that should not be exceeded. The political ideal would be to persuade the Soviet Union to withdraw its SS-20s from within striking distance of Western Europe (perhaps to some observable quarantine in Eastern Siberia?) so that the deployment of the Pershing and cruise missiles could be postponed. But that objective entails a paradox: the most effective way of winning an understanding with the Soviet Union is to continue to insist that the deployment of the US missiles will be carried through. On past form, a compromise should then be possible about the middle of 1983.

Long before then, the Geneva talks must be put on a much more rational basis. Two sets of bilateral negotiations, about nuclear missiles of intermediate and strategic range, are being carried on independently of each other. The distinction is absurd. The SS-20s that now potentially threaten Western Europe could be used both in a limited nuclear war in which the United States was not directly attacked (or threatened) or in a strategic all-button conflict. Similarly (Soviet analysts will calculate), Pershings deployed in Europe would be assigned to a variety of targets in the Soviet Union, some of which would necessarily be integral to a strategic attack (no doubt retaliatory), which probably explains why the Soviet Union has reacted so strongly against them. In such a negotiation, the British and French

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governments should either be participants or effectively be represented.

For Western governments, the implications of this complicated situation are bound to be uncomfortable. In Europe, governments will have to be telling anyone who will listen that they stand by what was agreed in 1979, but that they will refuse house-room for the Pershing and cruise missiles once there is some kind of agreement at Geneva. They will have to give further hostages to electoral fortune by pointing out the inconsistencies in the Soviet Union's insistence that it must be allowed to negotiate from strength. In the United States, however, there is an urgent need that the Reagan government should change tack. More factual and less strident accounts of the Soviet threat would help the Helmut Kohls of this world, while the eagerness of members of the US Administration such as Mr Caspar Weinberger, the Secretary of Defense, to prove that the Soviet Union is "not serious" about arms control is increasingly an impediment to a successful negotiation. Western governments in 1983 should try to persuade their own people that they are equally serious in their intentions both to increase the nuclear striking power in Europe and to negotiate a deal with the Soviet Union.

Max Born's centenary

Quantum mechanics is less than a century old; Max Born belongs to an earlier period.

The accompanying photograph of the late Professor Max Born is a monument to the heroic days of modern physics. Born was born almost exactly a century ago, and his anniversary has been widely celebrated in West Germany in the past few months, the fact of his having been driven out in the 1930s notwithstanding. Max Born is still widely revered as a perceptive and sympathetic teacher, as well as the author of an important textbook on atomic physics. Some may also know that with Jordan and, later, with Heisenberg, he contributed to the construction of quantum mechanics, still the most surprising theoretical construct of the twentieth century. Born's achievement, in the early 1920s, was that or reconciling in his own mind the notion that quantum states can be likened to vectors in some n-dimensional or even infinitely dimensional space, and physical quantities to operators in that space, a triumph of logic over intuition. That Schrödinger eventually stole the show was a kind of accident. And, in any case, Max Born eventually taught physics at the University of Edinburgh as if he had never been a hero.

Can there be excess TV?

Britain has changed from having too little to having too much television.

A few weeks ago, the British were widely regarded as a televisually deprived people, stuck with a meagre ration of three television channels, two run by the British Broadcasting Corporation and one other operated on behalf of commercial interests by a public corporation called the Independent Broadcasting Authority (IBA), financed by those given a licence to broadcast television signals accompanied by advertisements. The late Lord Thomson's remark that a television franchise was a licence to print money has ever since stuck in the public mind and has also been a challenge to the statutory broadcasting authorities: how can the entrepreneurs be kept in their tradesmen's places (chairmen of commercial television companies do not automatically qualify for knighthoods in the British system) while being cajoled into producing better television broadcasts than those that the marketplace would require?

The simple answer, so far, has been to let them make profits, but to take away the cream by means of a levy over and above taxation. More recently, IBA has required that profitable television contractors should contribute towards the cost of an extra television channel. Now, however, the broadcasting authorities are in a fix. The British government, more by accident than design, has decreed not merely that there should be at least two and possibly four extra television channels by 1986, but that there should be a national cable television system even sooner. And then, further to complicate the relief of visual deprivation, the British government has launched a fourth conventionally broadcast set of television signals among the British people, called Channel 4 by English-speaking people, but Canl Pedwar Cymru among those in Wales who are able, linguistically, to insist on speaking Welsh.

The immediate (but admittedly anecdotal) consequence of this development has been that people recently crying for more televisual choice have taken to complaining that they cannot possibly keep up with the output of four channels. Merely reading the menus on offer each day is a sufficient chore; actually watching what is on offer would be death of a kind. This, no doubt, is the reason why Channel 4 has so far been a disaster. It seems to have won the allegiance of a mere four per cent of the British televiewing audience, chiefly (it appears) on the strength of ancient films from Hollywood. What the new channel has so far failed to accomplish is the trick of making what it has to show overwhelmingly captivating for some minority among that section of the population at risk of having no other television signal at which to look. Why, in other words, has not the British Channel 4, the first national television network anywhere to be known by a number greater than three, failed in its first few weeks to live up to the ideals of its prospectus? Because it has not had the courage of its conviction that minority interests should be catered for. Science, for example, has been dealt with shabbily on the fourth television channel. On present form, the sixty-fourth channel will be no better.

The moral for the British broadcasting authorities, the British Broadcasting Corporation and the Independent Broadcasting Authority, is that they should practise what they preach, and in particular agree that as the opportunities for people to choose what they watch expand, the need that statutory authorities should oversee what people watch will inevitably decline. It is understandable but nonetheless unforgivable that the broadcasting authorities should resent the replacement of four by sixty-four channels of video signals. What they do not yet appreciate is that their present audiences are not disloyal but merely dissatisfied; they would like more choice, even if in the end they choose to watch what they have always watched, or nothing at all. If the market will bear that, is there any reason why the customers should be denied?

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US Public Health Service faces axe

Schweiker urges rethink on OMB cuts

Washington

The Office of Management and Budget (OMB) is proposing broad cuts in health-related programmes for the budget for fiscal year 1984. The National Institutes of Health (NIH) and virtually every public health agency would be affected.

The proposal is contained in OMB's preliminary draft of the President's 1984 budget, which is supposed to be secret until the final budget is published in January. Confidential documents detailing OMB's plans for the health programme, together with a strong protest by Health and Human Services Secretary Richard Schweiker, are now, however, to hand.

According to the documents, OMB wants to limit the NIH budget to $4,013 million; after inflation, this represents a cut from the current level of $3,954 million. The short-fall could be made up by "various 'grant-stretching' mechanisms", OMB suggests, such as reducing direct costs of grants by 4 per cent to 20 per cent, cost-sharing with grantees and limiting "excessive personnel compensation". NIH director James Wyngaarden is known to be concerned about the last of these; NIH are at present conducting their own study of the growing proportion of research money used for principal investigators' salaries.

OMB also wants to revive two old and controversial cost-cutting proposals. One is a reduction in the indirect, or overhead, costs that NIH pay universities receiving grants. These payments go for fixed operating costs, such as office space. NIH last spring provoked a storm of protest from the universities when they proposed cutting indirect cost reimbursement by 10 per cent. Congress has since attached language to the NIH appropriations bill forbidding any such cuts. The other proposal is that NIH should charge patients at their clinical centre for routine hospital costs.

Schweiker's appeal against the OMB plan asks for an additional $229 million for NIH, which would allow the institutes to maintain their present level of 5,000 grants and 10,000 traineeships. The OMB cuts would reduce this to 3,200 grants.

The other Public Health Service agencies appear to fare worse under the OMB plan. The Food and Drug Administration (FDA) would have its budget frozen at the 1983 level and in addition would be required to eliminate 240 full-time positions. The Center for Disease Control (CDC) would have to fire 760 of its 4,162 full-time employees and reduce its budget by nearly one-third, from $318 million to $215 million. The appeal argues that "unless these funds are restored, CDC would lack the capacity to deal with emerging health problems" and would have to "limit research on controlling the 10 leading work-related health problems". It also takes exception to OMB's assumption that FDA could raise $5 million by charging industry for the cost of processing new drug applications; FDA has not yet determined if "user charges can be collected without compromising the mission of the agency", the appeal says.

Schweiker is asking that OMB should add $35 million to the FDA budget and allow the agency to maintain its current staff level, and to limit the CDC cuts to $54 million and 60 full-time positions.

Schweiker is also protesting against organizational changes that OMB included in its budget plan. The most drastic of these would diminish the role of the assistant secretary for health, Dr Edward Brandt, to that of a policy adviser. Brandt, who is considered a powerful advocate of health issues, has operating authority over all of the Public Health Service agencies, including NIH, FDA, CDC and the Alcohol, Drug Abuse and Mental Health Administration; in addition, health promotion and environmental health programmes and the National Centres for Health Statistics and for Health Services Research are run directly from his office. The OMB plan would parcel out these programmes to the various agencies and would eliminate Brandt's authority over the agencies. In effect, the Public Health Service structure would be abolished; each of the agencies would report directly to Secretary Schweiker.

One White House source said "I think Schweiker will be successful in appealing these massive organizationl changes which destroy the Public Health Service". The disposition of OMB and the President towards his other appeals is unknown, although there is strong support for a real increase in NIH funding within the President's Office of Science and Technology Policy.

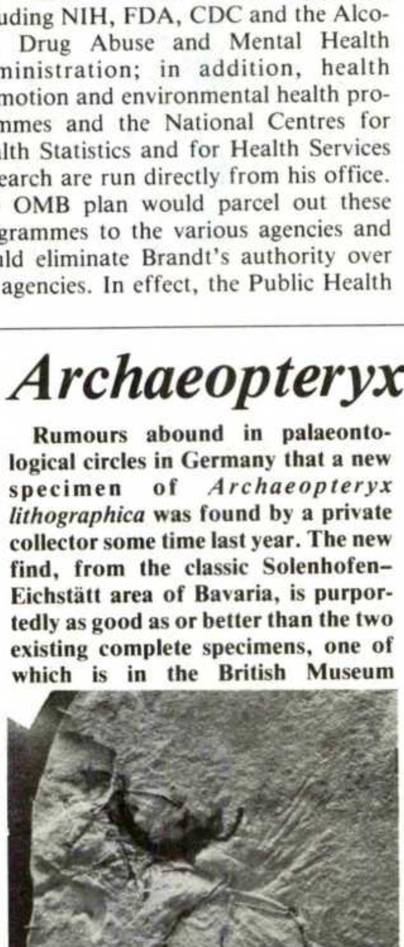
If OMB's proposed cuts do survive Schweiker's appeal, they still face what is likely to be strong opposition in Congress. "Congress would not be very enthused about any of them", said a staff member of the House Appropriations Committee. The support for health research in Congress was demonstrated earlier this year when Congress added $312 million to the Administration's $3,642 million request for NIH.

And a staff member of the House Energy and Commerce Committee, which oversees the Public Health Service, pointed out that the President's budget has tended to carry less weight in recent years owing to the increasing use of continuing resolutions to provide funding. This mechanism is invoked when the new fiscal year arrives and the agency-by-agency appropriations bills have not yet been passed. Continued spending authority is lumped into a single stop-gap bill which a President must sign, or the government ceases operations. This restricts the President's power to impose his version of the budget on Congress.

Stephen Budiansky

Archaeopteryx in hiding

Rumours abound in palaeontological circles in Germany that a new specimen of *Archaeopteryx lithographica* was found by a private collector some time last year. The new find, from the classic Solenhofen–Eichstätt area of Bavaria, is purportedly as good as or better than the two existing complete specimens, one of which is in the British Museum (Natural History) where it is undergoing a further round of extensive preparation.



Of the five known skeletons of *Archaeopteryx* (the sixth example is a single perfectly preserved feather) the "Maxberg exemplar" (see left) found in 1956 is the least known, as the owner is loth to allow specialists even a glimpse of it and only a preliminary description has ever been published.

Whereas the neighbouring state of Baden–Württemberg has legislation which prohibits the sale of finds of scientific importance without special museum authorization, in Bavaria, where no such legislation exists, it is a case of finders-keepers or sale to the highest bidder. And with prices in the hundreds of thousands of deutschmarks for Pterosaur speciments, what price an *Archaeopteryx?*

Mike Howgate

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Soviet science and technology

Andropov has his say

The Soviet Union will spend 25,500 million rubles on scientific and technological research next year — an increase of 6.2 per cent on 1982. The publication of actual investment figures is somewhat rare in the Soviet media, which prefer to cite increases in percentages only (often referring to inaccessible data-bases). The announcement of this increase — which in real terms represents a rise of some four to five per cent, since inflation in the Soviet Union is running at between one and two per cent annually — was made in a *Pravda* editorial (a traditional Soviet method of announcing a new policy drive) dealing with the speech of Party Secretary Yurii Andropov at last month's plenum of the Central Committee of the Communist Party of the Soviet Union.

According to Mr Andropov, the "great reserves" available to the Soviet Union need to be used "more actively and more consistently". This is basically a reiteration of the Brezhnev policy of modernizing industry by high-priority implementation of research results in industry. This policy, Mr Andropov said, would be continued with even greater impetus — evoking from Academician Anatolii Aleksandrov, president of the Academy of Sciences, the rejoinder that if research results were not being implemented on schedule it was not the scientists but the production ministries which were at fault.

Mr Andropov, however, has some ideas of his own as to how this policy should be pursued. Already the Academy of Sciences carries major responsibility for co-ordinating the research programmes launched to meet the specific needs of Soviet industry and agriculture. Now, it appears, in addition to producing new equipment and "front-ranking work methods", the academy is set to take on a troubleshooting role. Together with the State Committee for Science and Technology and the relevant production ministries, it will be expected to "pinpoint and eliminate" the difficulties hindering technical progress. This approach could be of considerable importance for research planning.

During the past ten years, there has been emphasis on the establishment of local "science centres" (usually created by linking existing institutes in the locality), which were intended to serve the research needs of local industry, frequently on a direct contractual basis. The initiative was normally left to the industrial side, which identified production problems and then sought scientists' help to solve them. The new procedure suggests that scientists will now be expected to identify problems as well as solve them — a procedure which would not necessarily appeal to all enterprise directors. Retooling and redesigning of the production line is a nightmare for Soviet management, since the regular quarterly, annual and quinquennial targets make no allowances for temporary shutdowns. A zealous scientist urging such a closure in the name of long-term improved efficiency might well run foul of a director eager to meet his immediate target figures.

Perhaps to overcome such frictions, the recent plenum urged the importance of material incentives for those involved in modernization projects. People who "boldly go for the introduction of new technology", it noted, should not find themselves placed in a "disadvantageous situation".

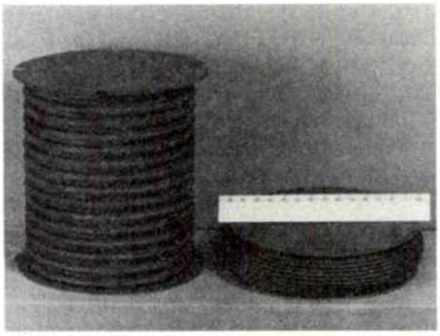
Vera Rich

Nuclear waste disposal

Synroc presses on in Australia

The Synroc programme — Australia's synthetic rock alternative to glass for the disposal of highly active nuclear waste — is about to move up a few gears with the construction of a cut-price, "commercial-scale" Synroc plant 20 miles south-west of Sydney. The plant, at the Lucas Heights Research Laboratories of the Australian Atomic Energy Commission (AAEC), will be big enough to cope — in principle — with 600–900 tons of spent fuel a year. But in practice it will not see a jot of nuclear waste.

The objective, according to Dr Keith Reeve, leader of the Synroc project at AAEC, is nevertheless to prove that Synroc is a practical alternative to glass. Tenders closed last week for contract management of the detailed design work, and, says Reeve, the successful bidder is likely to have considerable experience of nuclear plant — for AAEC and the Australian National University (ANU), which is also involved in the project, wish to build a plant which, although non-radioactive, is compact, easily maintained, and demonstrates that active Synroc could be made safely.



A recent 30-kg block of Synroc compressed using the uniaxial bellows hot pressing technique. Primary minerals (and waste) are sealed in evacuated stainless steel bellows, and pressed and heated without side supports. The resulting blocks would then be sealed in a larger can.

Cost precluded the construction of a fully active plant, according to Professor Ted Ringwood, inventor of Synroc and ANU representative on the demonstration plant project. A remotely-controlled active plant might have cost "hundreds of millions of dollars" (A$1.7 = £1), says Ringwood "of the same order as a glassification plant". But AAEC and ANU have only A$2.7 million to spend, the amount of a government grant for the project, plus staff support from AAEC and ANU. A little over A$1 million was released in August, with the rest promised over the next two years, for construction in 1983–84 and operation in 1984–85. "I blow hot and cold on whether these dates are optimistic or pessimistic", says Reeve.

Whenever the plant is ready, it will make Synroc-C, which consists of 60 per cent titanium dioxide, with an admixture of barium oxide, calcium oxide, zirconia and alumina; these react at 1,100–1,170°C and 100–400 atmospheres pressure to create three mineral phases related to perovskite, hollandite and zirconalite. These phases can absorb up to 20 per cent weight of highly active waste oxides, according to Reeves.

At AAEC in Lucas Heights, experiments have concentrated on producing the Synroc in large-scale blocks, rising from a few granules in 1979 to 30-kg hot pressings by the end of 1981. The Synroc itself is able to retain foreign elements much better than glass, the Synroc team maintains; it is also 59 per cent denser and can withstand higher temperatures.

The apparent weakness of the Synroc programme, however, has been the relative lack of work on active Synroc — Synroc actually containing nuclear waste. "We're sensitive to that criticism", says Reeves and in fact next year Reeves plans a "two line" radioactive test programme. One wil involve neutron-irradiated uranium oxide (to stimulate fission products) in smal (½–1 cm diameter) Synroc samples, on which leach tests will be done. The oxide will be fresh from irradiation, Reeve stresses, unlike spent fuel which has been cooled for a few years, and it will b possible to reproduce in two years the tota beta-ray dose expected in a piece of active waste-containing Synroc over its lifetime The samples will then be subjected to leach tests. Alpha irradiation will also b simulated with neutrons.

And in the second line of radioactiv tests, Synroc will be made in a glove bo with "trace" levels of actinides – neptunium, plutonium, americium an curium. The work will start in June.

Robert Walgat

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West German research and technology

Meeting of past and present

The ceremony last week to open the new London premises of the Anglo-German Foundation for the Study of Industrial Society was attended by the unlikely trio of Dr Heinz Riesenhuber, the present minister at the West German Ministry for Research and Technology (BMFT) and two of his predecessors, Dr Andreas von Bülow and Professor Hans Leussink.

Dr Riesenhuber was in London to discuss Anglo-German cooperation in science and technology with the British government, especially in the context of international organizations such as the European Molecular Biology Laboratory and in information technology. He is also interested in joint industry–government collaboration between Britain and West Germany.

Such work might be managed on the West German side by the federally supported Large Research Centres. Riesenhuber reiterated his aim of directing the work of these centres to more industrial projects and his hope that small to medium-sized firms (fewer than 200 employees) will band together to support research at the centres. This may simply mean that these centres impinge on the ground at present occupied by the Fraunhofer Gesellschaft, which supports a number of cooperative research laboratories, and independent contract research centres without increasing the amount or effectiveness of research and development. It remains to be seen, however, what form the as yet undefined "indirect" support of industrial research will take.

Heinz Riesenhuber . . . advocate of collaboration

Last week the minister acknowledged the achievements of West German science under his predecessors and, conscious that research and development is a long-term activity, accepted that change must come slowly. He is, however, concerned about the mediocrity of much basic research and wants to give more support to outstanding scientists. In a constant budget for basic research, this means that money will be less evenly spread. On the other hand, it is not clear how budding geniuses are to be identified before they flower, and with the proposed replacement of student grants by loans (even in secondary education) from 1984 those from modest backgrounds may find it difficult to be recognized. Nevertheless, Dr Riesenhuber believes he knows who the top five in genetic engineering are, and hopes to lure at least one of them to a Large Research Centre.

Dr von Bülow, Riesenhuber's immediate predecessor, was present in his capacity as deputy chairman of the trustees of the Volkswagen Foundation, which has paid for the renovation of the premises taken over from the Pharmaceutical Society. Since leaving office in October, Dr von Bülow has made known his belief that one of the two experimental nuclear reactors should be abandoned. (The costs of completing the fast and high-temperature reactors were a persistent problem for him as they still are for his successor.) In von Bülow's view, the price of uranium is such a small factor in the overall cost of existing light-water reactors that neither reactor can become competitive in the next thirty years, whatever the price of other fuels. This is in sharp contrast with the policy of the present government to complete both reactors. **J.S. Dunnett**

Polish education

New students' association's aims

Poland's new student union, the Association of Polish Students (ZSP), launched last month, has announced a wide programme of action and campaigning to cover virtually all aspects of the higher education process — "the formulation of demands concerning secondary education", equal opportunities of entrance to university, grants, rest and sports facilities, the patronizing of student artistic activities and the "student scientific movement", rationalization of the job market and a loans system for graduates who have difficulties in obtaining housing.

Just how ZSP intends to pursue these aims is not clear — under the new Higher Education Act, which came into force on 1 September, student associations do not have trade union status, and the right to strike is denied them. Some innovations — such as student grants, means-tested on the income of the whole family — have already been introduced by the new act. Other problems, including the disparity of secondary educational standards between urban and rural schools, are already matters of serious government concern. One of the "most important goals" of the association, "exerting influence on matters concerning further education" and the creation of "self-management and democracy" within universities and colleges, is, the statute admits, already covered by the new law, and the association's role will be to lobby for improvements and amendments.

ZSP was founded as a continuation of the old Socialist Association of Polish Students (SZSP), which lost most of its members in autumn 1980, when the Solidarity-type Independent Students Association (NZS) was founded, while many of its functions, including travel and welfare matters, were taken over by "self-government committees" on which both unions were represented. After NZS was banned last January, its members showed no sign of returning to SZSP. Accordingly, shortly before the SZSP Congress (19–20 November), the governing council of SZSP recommended that the word "Socialist" should be dropped, and that the association should be reshaped to draw in as wide a membership as possible. At the same time, the governing council made clear, the experience of the past ten years should be incorporated and a firm commitment made to socialism. Thus the new ZSP statutes not only acknowledge the "leading role" of the party but also pledge the association to fight for the implementation of socialist values in Poland, and to cooperate with "progressive" youth organizations abroad in the "battle for peace and national and social liberation".

The Congress of SZSP/ZSP met against a background of continuing university unrest. A few days earlier, all teaching in Torun University had been suspended for a week after disturbances in one of the halls of residence. At Warsaw University, the department of psychology was closed for three weeks pending an inquiry by a special "Rector's Commission" into the stoppage of lectures and classes on 10 November, the Solidarity "day of action". (Few universities came out fully in support of this "day", although token stoppages and meetings were reported in several universities, including Krakow and Wroclaw; in Warsaw, there was a rally on the main campus of the university, estimated by the party newspaper *Trybuna Ludu* at around 1,200 persons.) The Warsaw psychology department was allowed to reopen last week following the resignation of the dean and sub-dean, although many students still face disciplinary proceedings.

How far the new association will go to help defuse the situation remains unclear. One innovation proposed for the new ZSP seems to have been designed to provide a ballast of older members, who could well act as a calming influence. In addition to the ordinary student members, ZSP is to admit "supporting members" — Polish citizens who support the statute and who take some active part in the work of the association. **Vera Rich**

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Stanford graduate students

Clampdown on external links

Washington

Members of the Stanford University faculty will not be allowed to employ their graduate students in jobs in outside companies under a new policy adopted last week by one faculty.

"We felt that this was an area that could be abused", said Dr Gerald Lieberman, dean of graduate studies and research. The concern is that a faculty member may in effect make employment in his outside firm a condition for taking on a graduate student. The new rule forbids employment by a faculty adviser or by a member of a student's dissertation committee. The Massachusetts Institute of Technology has a similar rule.

William Smith, a member of the Graduate Student Association's academic affairs committee, said that he knew of one case where such employment arrangements may have been abused. A graduate student not involved in his research adviser's outside company filed a complaint with the university ombudsman that a fellow student, who was employed by the company, was receiving preferential treatment from the adviser.

The effect of the new rule is unclear. Neither Smith nor Lieberman could say how many students throughout the university are employed by their faculty advisers in outside ventures, although Smith said he knew personally of "about ten" students in that position. But he also said that in any case the rule "won't change anything", because of a loophole that allows exemptions to be granted by department chairmen, so that in effect the rule will be little more than a reporting requirement.

Lieberman, though, said "it puts the burden of proof on the faculty member — I think it's a psychological barrier".

A more controversial proposal will be taken up by the faculty's committee on research this winter; this proposal — recommended by the graduate student association — would require annual reporting by faculty members of all outside activities. A similar requirement was recently proposed by a faculty committee at the University of California at Davis, stemming in part from the controversy surrounding Professor Ray Valentine's involvement with his own company and Allied Chemical that surfaced last spring (see *Nature* 4 March, p.6)

Many members of the Stanford faculty reportedly believe such a requirement to be an intrusion of privacy; Lieberman noted that "the big disadvantage is the feeling that once a faculty member has reported, he's absolved of 'guilt'. That means that if you have reported, someone has to take it seriously", which means reviewing the reports and following up on them where necessary. He questioned whether that would be done.

In the recommendation from the graduate student association, Smith writes that at least ten undergraduates, graduates and postdoctoral students have lodged complaints about conflicts of interest on the part of their advisers that they believed interfered with their education and training. The problem, Smith said, centres in the departments of biochemistry, chemical engineering, computer science, electrical engineering, mechanical engineering and medical microbiology.

Stephen Budiansky

UK nuclear power

Hold on fast reactors

The British government's decision to put its fast reactor programme on ice, made public last week in the House of Commons, has spread consternation throughout the UK Atomic Energy Authority. Although the research establishment at Dounreay in the north of Scotland is not likely to be much affected by the decision, other research establishments at Risley (Lancashire), Harwell (Oxfordshire) and Winfrith (Dorset) are likely to suffer reduced budgets in the years ahead. So too is the team of design engineers maintained by the National Nuclear Corporation on the Risley site, which has been putting the finishing touches to the design of a full-scale fast reactor for the past several years.

According to Mr Nigel Lawson, the energy secretary in the British government, it has been decided that the electricity generating industry will not on economic grounds need to order fast reactors before the early years of the next century, so that the programme of fast reactor development based at Dounreay can prudently be stretched out.

The Atomic Energy Authority has therefore been asked to put forward a revised development programme, taking account of the new time-scale for the commercial application of fast reactors and of the opportunities for international collaboration on such projects. A spokesman said this week that it was hoped to have a new fast reactor strategy by the early spring of 1983.

By all accounts, it has been decided to keep the Dounreay reactor working, chiefly so as to try out novel kinds of uranium and plutonium fuel and to test the efficacy of the pilot-scale reprocessing plant in operation there. The extent to which the Dounreay establishment will become the focal point for research and development on fast reactors will depend on the readiness of others among the authority's establishments to give up their interests in the subject.

In the present programme of research and development by the Atomic Energy Authority, fast reactor work acounts for an annual expenditure of about £100 million. Of this sum, roughly a half is spent in the north of Scotland. Part of Mr Lawson's calculation seems to be that if the authority is told clearly that the programme must be stretched out, the result may be more diligence in looking for partners elsewhere. It seems also however to be understood by those concerned that the National Nuclear Corporation should have a say in the final decision on whatever development strategy is eventually agreed.

The outcome of that process is likely to be uncomfortabe for the authority's research establishments. The chances are that, in the spring, the government will be looking to the authority for savings of about a quarter of its current expenditure on fast reactor development. (It is unlikely that even the present government will follow the opinion of some officials of the National Nuclear Corporation that the Atomic Energy Authority's own future should now be reconsidered.) The best solution would be a deal with some comparable authority elsewhere that would postpone the evil day until fast reactors are clearly economic. ●

Dutch scientific research

Good and bad

Waalre, The Netherlands

The Netherlands' two major scientific research organizations have experienced mixed fortunes during the past week. While the Netherlands Organization for the Advancement of Pure Science (ZWO) was finalizing plans to become the first foreign partner to share the British Synchrotron Radiation Source at Daresbury, on the home front the Dutch national applied science organization, TNO, was announcing 550 job losses as a result of a declining government subisidy and reduced orders from government ministries.

On 2 December Professor W.F. de Gaay Fortman, chairman of ZWO, and Professor John Kingman, chairman of the UK Science and Engineering Research Council (SERC) signed an agreement which means Dutch scientists will have access to the Synchrotron Radiation Source at SERC's Daresbury Laboratory. In addition the Dutch partners are to construct a new beamline at Daresbury in 1983–84, fitted with experimental stations to carry out extended X-ray absorption fine structure (EXAFS) and small-angle diffraction (SAD) studies (see diagram). The Dutch share of construction costs will be around £1 million, in exchange for which the British will provide local support for visiting Dutch scientists as well as the

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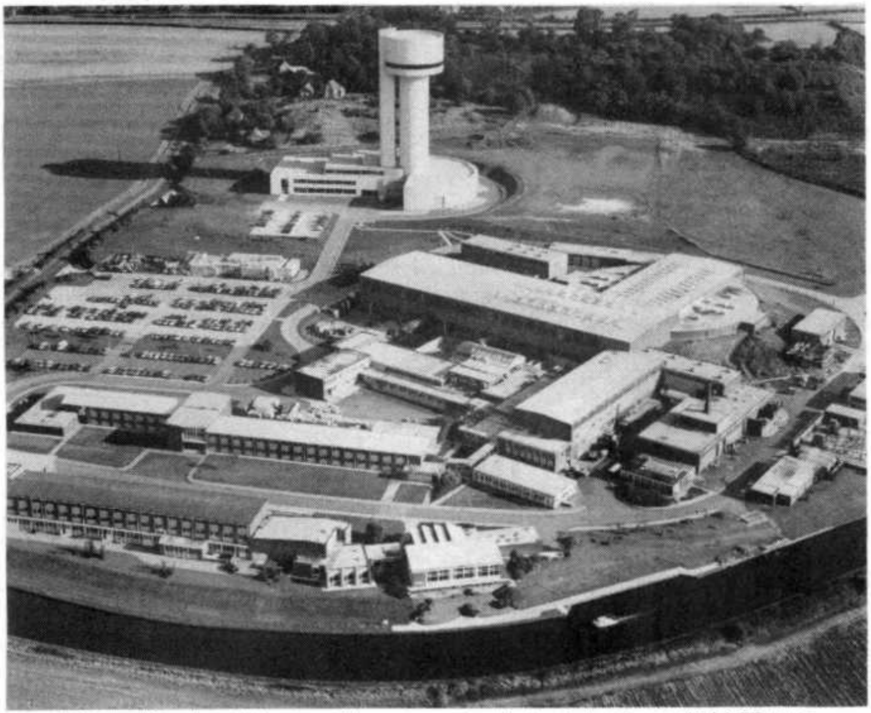
experimental facilities.

The collaboration on the Daresbury facility is the first fruit of an "umbrella agreement" signed between ZWO and SERC in June of this year to promote scientific collaboration between the two countries. Previously a protocol on collaboration in astronomical research was signed in La Palma in June 1981 and the two countries are also cooperating, together with the United States, on the Infrared Astronomical Satellite (IRAS) due to be launched on 27 January 1983.

The number of experiments being performed at Daresbury will double during the next few months. The deal with ZWO is one source of the increased work load, but cooperation between SERC and industrial concerns is also on the increase. A consortium of Shell, ICI and British Petroleum is already working with SERC and Professor Kingman hopes that "more money but also more work" will be forthcoming from industry in the near future. Kingman is also optimistic that further cooperation with the Netherlands will follow within the framework of the recent mutual statement of scientific collaboration.

The industrial sector too is the one small sign of hope apparent in the recent announcement from TNO. Despite an increase in orders from industry, government cutbacks have resulted in an increase in TNO's annual deficit from 7 million guilders in 1981 to an expected 15 million guilders in 1983. TNO is to lay off 350 workers in the next four years and a further 200 vacancies will be left unfilled — this represents a cut of around 10 per cent of the staff of 5,000. Research priorities are to be adjusted in response to the crisis, with less expenditure on nuclear energy but more on energy conservation, less materials research and better integration and reorientation of environmental and health research, all politically welcome goals.

Casper Schuuring



An aerial view of Daresbury Laboratory. The most prominent feature is the 72-metre high NSF tower housing one of the world's highest-voltage Van de Graaff accelerators.

Satellite broadcasting

Digital Britain

The British Goverment, not often distinguished by its openness, seems nevertheless to be ready to tell more than the whole truth about the technology of its plans for the eagerly awaited communications revolution. Last week, the Department of Industry issued a formal recommendation by a technical committee of the standards that should be adopted in the design of direct-broadcasting satellites. The first of these is expected to be in service in the United Kingdom in 1986.

As if to emphasize that the traditional technology of the communications industry has been superseded, the report, which runs to 54 pages, is on sale at Her Majesty's Stationery Office (Cmnd 8751) at a price of £5.20 — almost £0.10 a page.

Technically, the recommendation of the advisory panel (chairman, Sir Anthony Part) is however surprising by its daring. The committee has rejected the technical proposals of the British Broadcasting Corporation (BBC), which will operate the first two of the five direct broadcasting channels available to the United Kingdom.

The BBC, in evidence to the committee, had argued for an extension of present analogue codes for specifying the intensity and colour of picture elements on a television screen. In effect, the BBC's proposal would have been a more sophisticated version of the widely-used PAL system in which the transmission of colour information is confined to a small part of the available band width. The basic PAL patent expires in 1983.

In the event, the committee has plumped for a digital system for transmitting television signals. The system concerned, although devised by the Independent Broadcasting Authority, has been offered free of charge to the BBC. What seems to have swayed the advisory committee is the technical superiority of the new system (known as MAC) and the chance that it might subsume both PAL and SECAM, the two broadcasting standards at present used in Europe.

Royal Society

Break ground

This year's annual report from the Royal Society of London, most notably subversive because of what it says on relations between British and Soviet scientists (see *Nature* 3 December), also describes how the society has polled its fellows in the hope of finding out how their universities are surviving and how it is moving (gingerly it seems) towards an assessment of the consequences of nuclear war. The Royal Society, it seems, is on the move, although its direction is as yet by no means clear.

The society's annual report explains that a questionnaire was sent out in June to all members of the society, asking for comments on conditions in their subjects — not merely in their universities. The general response seems to have been a complaint by established academics that it is no longer possible, in the British university system, to appoint promising young people to permanent or even temporary posts. Replies to this questionnaire seem to have been influential in persuading the Royal Society to urge on the British government the need to recruit more young people to academic posts.

The society has also broken new ground by its decision to contribute to public discussion of the consequences of nuclear warfare. The annual report says that the annual meeting in November 1981 was partly given over to a discussion of this topic, but that the society's council has not yet decided whether to mount a formal study of the question. The significance of that development is merely that it would, if carried through, be entirely without precedent.

For the rest, the Royal Society has a disappointing tale to tell of its success in spending funds on exchange visits by scientists. In relationship to Western as well as Eastern Europe, it seems, the willingness of British scientists to travel has declined. A new scheme to enable exchanges with Australasia may nevertheless take up the slack.

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UK manufacturing

Cambridge Instruments upturn

The British-based Cambridge Instrument Co. Ltd (CIC) appears successfully to have weathered the storm that almost drove it out of business three years ago. The latest half-yearly figures show the company to have made a remarkable return to profitability. The company now hopes to capture a major part of the future market for robotic vision systems and for manufacturing equipment for gallium arsenide semiconductor devices. An average annual loss of £3 million in the three years to 1979 has been converted to a pre-tax profit of £1 million on a £22.7 million turnover in the year ending 31 March, and profits for the first half of the current year are double those for the same period last year.

This dramatic change in the company's fortunes is attributed by people in the company to the management skills brought in by Terence J. Gooding, a "company doctor" from the United States who bought a substantial shareholding in 1979. As the Cambridge Scientific Instrument Company, CIC was in the early 1960s the first to produce a commercially viable scanning electron microscope, and this type of equipment remains the largest part of the business. In the late 1970s, however, the company appeared to some to be resting on its laurels and to have lost sight of some commercial realities; it produced a scanning electron microscope which was far in advance of its competitors in specification but which was also so expensive to build that it could not be sold profitably.

The lessons of that expensive error seem to have sunk in. Now, the company is concentrating on feeding back information about customers' requirements to the development teams and is once again increasing its share of the market for scanning microscopes. As an example of customer-inspired development, the company cites its new large-chamber microscopes, used in the semiconductor industry for the examination of large wafers. Now, a US factory is planned to cope with increased demand.

The company has also recently introduced new products in its range of electron-beam microfabricators. The new system, called "Chiprite", promises to double throughput in integrated circuit manufacture, with very high resolution. Together with what the company calls a "revolutionary" new integrated circuit checking system, CIC hopes by these means to expand its already large share of the market for semiconductor manufacturing equipment.

Robotic vision, on the other hand, seems to have arisen as a spin-off from the existing technology of image analysers. By the simple expedient of fitting these machines with zoom lenses, CIC finds itself able to apply existing software to the control of industrial production processes.

Another major market that CIC hopes to exploit is that for machinery for the manufacture of large crystals of gallium arsenide. This semiconductor has the substantial advantages over silicon that circuits can be packed more densely, partly because of the lower power dissipated, and that switching times are up to ten times faster.

Gallium arsenide is at present used largely in military applications, but civil applications are likely to flourish in the coming decade, in satellite television links for example. Gallium phosphide and indium phosphide, two other potentially important semiconductor materials, are also produced on CIC equipment. In this area, the company claims the lion's share even of the coveted Japanese market.

Tim Beardsley

Technology drive

Korea

South Korea is to launch an "aggressive drive" to upgrade its technology to international levels. Reporting to a recent technology promotion conference, chaired by President Chon Tu-hwan, the Science and Technology Minister Yi Chong-o described his ministry's new eleven-point plan, which includes the introduction of foreign technology on a "new scale", the promotion of joint research and development projects by Korean institutions and their foreign counterparts and the encouragement of the private sector to set up small and medium-sized technology-intensive corporations in advanced countries. An "active recruitment" programme will be launched by the ministry to encourage South Korean scientists now working abroad to return home and take part in the technology drive.

Vera Rich

Heavy ion research

France finds niche in GANIL

French heavy-ion physics took a step forward on 19 November when GANIL, the national heavy-ion accelerator at Caen in Normandy, produced its first beam. The accelerator's two principal cyclotrons were coupled and, with the help of a smaller injection cyclotron, accelerated a beam of argon ions to 1.8 GeV (45 MeV per nucleon). The first experiments will begin in January.

The decision to build GANIL (Grand Accelerateur National d'Ions Lourds) was taken in 1975 by the then industry minister, Michel d'Ornano. The project was delayed by an early lack of finance but the now completed facility remains within the initial cost estimates of about FF500 million at current prices. According to the director, M. Claude Detraz, the first experiments should start on 10 January; six months' worth of experiments have been planned.

According to its director, GANIL will carry physicists into an intermediate energy and intensity range of unique interest, making it in some ways complementary to other leading heavy-ion accelerators at Berkeley in California, Dubna (near Moscow) and Darmstadt. The Berkeley Bevalac accelerator is capable of accelerating the heaviest nuclei to energies of 1,800 MeV per nucleon. At such relativistic energies, according to current theories, the 3-quark bags that make up each of the protons and neutrons in the nucleus will, it is hoped, break up, leaving quark matter. At lower but still relativistic energies, collisions between heavy nuclei involve interactions between their constituent nucleons. Models for such collisions are comparatively well developed in West Germany.

The Gesellschaft für Schwerionenforschung (GSI) at Darmstadt in West Germany devotes much of its efforts to lower energy regimes, near the Coulomb barrier at 6–7 MeV per nucleon. In this regime, the nuclei undergoing collisions are left in internal equilibrium. By careful experimental arrangement, the heavy nuclei can be made either to rotate at very high frequency — thus allowing investigation of newly discovered angular momentum states — or to collide gently and form new superheavy elements, the most recent being element 109, which was first synthesized a few months ago. (The accelerator at Dubna is also devoted to such experiments.) Successful models have been established for collisions at these energies that treat the nuclei as "collective" bodies.

GANIL will concentrate on the energy regime between GSI and Dubna, producing sufficient intensity (10^{13} nuclei per second) for rare species such as neutron-rich nuclei far from stability to be investigated (such as ^{20}C). In the next six months, ions of neon, argon and krypton will be accelerated at all energies (the highest for Ar, for example, being 80 MeV per nucleon). Preliminary experiments at CERN in Geneva have given some idea of what may be expected. For instance, there seems to be a limit to the amount of linear momentum that can be transferred to the target nucleus as the beam energy is increased. The reasons for this phenomenon are not known. Such glimpses apart, however, GANIL will be taking nuclear physicists into unknown territory.

Philip Campbell

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Intermediate vector boson

No Christmas boon for CERN

The race to discover the intermediate vector bosons, the set of particles supposed to play the role of the photon in the weak nuclear interaction, is in full tilt this week, at the European Centre for Nuclear Research (CERN) near Geneva.

Experiment UA1, the larger of two experiments (a collaboration of 13 institutions involving 128 physicists), already has one or two candidates for the charged boson (the W), but the group cannot be certain yet whether these events are signal or background. The smaller experiment, UA2 (6 institutions and 51 physicists), is being more cautious, and wants time to consider its "public statement".

"Ho, Ho, Ho! You didn't think I'd forget?"

So it seems that unless phenomenal luck strikes the groups this week, and they observe a clear signal for a Z, the neutral boson which is easier to distinguish from background but ten times rarer in the experiment than a W, Christmas may come without the present that CERN director Professor Herwig Schopper was hoping for (see *Nature* 18 November, p.205).

There are two technical problems: low average luminosity and high background. The luminosity is a measure of the frequency of events — collisions between protons and antiprotons producing showers of particles, among which the bosons must be sought. When UA1 was designed, the expected luminosity was 10^{30} cm^{-2} s^{-1}, but the current figure is nearer 4×10^{28} cm^{-2} s^{-1}, 25 times lower. Thus in the total run-time this year, UA1 expects to have collected only about five Ws but to have only a 50 per cent chance of having collected a Z, needles in a giant haystack of the other particles produced in collisions at the collider centre-of-mass energy of 540 GeV (that is, approaching 600 proton masses of available energy).

Even with the strategy being followed at CERN for detecting the intermediate vector bosons, this background is uncomfortably high. The trick is to look for electrons with energy of 40 GeV emerging perpendicular to the direction of the collision.

The argument is as follows. If a boson, WNZ, of mass about 80 GeV were formed at rest in the centre of mass, it would decay into two leptons — electrons, neutrinos, or electron and neutrino — with roughly half the 90-GeV decay energy in each particle. These particles could emerge in all directions, whereas the predominant quark–antiquark interactions in collisions between a proton and an antiproton produce only glancing blows and hence little sideways movement.

The objective is thus to detect the sideways 40-GeV electrons. However, gluon–gluon collisions between the "colour" fields of the proton and antiproton can also produce tracks in the detectors which mimic such electrons, and the trick is to distinguish one from the other. In the case of the Ws, which produce only one electron (and one neutrino), this can be done only by distinguishing the average kinematics of the tracks. And of course, to make distinguishable averages, reasonably high numbers of events are necessary. The rarer neutral Z, however, decays into an electron and a positron, both of which should be detected, so making identification much easier.

Next year the search is to be stepped up, with a projected luminosity of 10^{29} $\text{cm}^{-2}\text{s}^{-1}$ and a three-month run — a plan causing some consternation among other users of the Super Proton Synchrotron (SPS), which was originally intended to fire high-energy protons at fixed targets. When the collider was proposed, there was a proviso that it would never take more than a third of total SPS run-time. Next year the ceiling is to be broken in an effort to get the intermediate vector boson: 43 per cent of experimental time is to go to the collider.

Robert Walgate

Franco-Indian deal

France and India reached a compromise on the supply of enriched uranium for India's Tarapur reactor near Bombay on 27 November — on the eve of a visit to India by the President of France which may result in major economic and military agreements.

Supplies for Tarapur were originally guaranteed by the United States in a 30-year agreement dating from 1963, and backed up by a tripartite inspection arrangement between the International Atomic Energy Agency and the two countries in 1971. But both deals were invalidated by President Carter's Non-Proliferation Act, which forbade the supply of sensitive materials to non-signatories of the international Non-Proliferation Treaty — of which India is one. Carter, who had forgotten — or ignored — the treaties with India, managed to override the act to supply one load of fuel; but President Reagan has felt unable to continue in this manner. France has accordingly stepped into the breach, but began by demanding inspection rights which Mrs Gandhi was unwilling to accept. These guarantees would have gone beyond the terms of the 1963 and 1971 treaties.

In Paris, it was being argued last week that it would have been more dangerous *not* to sign the present compromise agreement than to stand off and let India reject the 1963 and 1971 inspection terms altogether. India already has a considerable amount of waste fuel at Tarapur, which could be reprocessed to extract the plutonium whenever India felt morally free to do so, the justification goes. The present agreement preempts that possibility.

Robert Walgate

Nature index of biotechnology stocks

1982 high	1982 low	Company	Close previous month	Close 26 Nov.	Change
46¼	16⅛	**A.B. Fortia (Sweden)**	39½	46¼*	**6¾**
8	2	**Bio Logicals (Canada)**	2¼	4½	**2¼**
7	3⅝	**Bio-Response (USA)**	5	6½	**1½**
14⅛	7¼	**Cetus (USA)**	9¼	12⅛	**2⅞**
13⅛	6⅛	**Collaborative Research (USA)**	9¼	13⅛*	**3⅞**
12⅝	5¼	**Damon (USA)**	9⅛	12⅝*	**3½**
23½	8¼	**Enzo-Biochem (USA)**	17	23½*	**6½**
28	6⅝	**Flow General (USA)**	13¼	13⅛	**–⅛**
47¼	26	**Genentech (USA)**	47¼	40¼	**–7½**
9¼	7	**Genex (USA)**	9¼	9¼*	**½**
6¼	2¼	**Genetic Systems (USA)**	5⅛	6¼	**1⅝**
26¼	9⅝	**Hybritech (USA)**	20½	25¼	**5¼**
14	5	**Molecular Genetics (USA)**	9¼	14*	**4¼**
52	34⅞	**Novo Industri A/S (Den.)**	47½	45⅝	**–1⅞**
21	8	**Monoclonal Antibodies (USA)**	14¼	21*	**6¼**

The *Nature* Biotechnology Index for November 1982 stands at **157.4** compared with **140.2** last month. Base is 100 as of 25 June 1982. Previous indexes appeared in *Nature* 12 August, p.599; 9 September, p.101; 14 October, p.573 and 11 November, p.101. Where stocks are traded over the counter, the price quoted is the bid price For stocks traded on the American Stock Exchange and the New York Stock Exchange, the price quoted is the transaction price Data from E.F Hutton, Inc

* New high or low for this calendar year

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CORRESPONDENCE

Lead in petrol

SIR — Jasper Becker's report (*Nature* 4 November, p.6) on efforts to persuade the European Community to revise its directive on the lead content of petrol perpetuates a general misunderstanding about the directive by saying that it permits a maximum level of 0.4 grammes of lead per litre of petrol and a minimum of 0.15, thus implying that petrol with less than 0.15 grammes is not permitted. Later the report reinforces this misconception by describing the current campaign to introduce lead-free petrol and saying that "no member state is at present allowed to do this"

In fact the directive does not prevent anyone marketing lead-free petrol. What it does do is to prevent any member state from banning lead in petrol or insisting on any lead level less than 0.15 grammes per litre. Thus if the motor industry and the oil companies could agree, it would be possible for lead-free petrol to be introduced tomorrow. If, however, member states are to intervene and insist on lead-free petrol from a certain date they will have to do so simultaneously by agreeing an amending directive.

Another requirement of the directive that deserves to be better known is that the member states have to supply the EEC Commission, at its request, with information on the "development of the concentrations of lead and polluting substances in the urban atmosphere and their effect on public health". The Commission in turn is to report to the Council and Parliament on the information thus obtained and, in the light of the data compiled, is to make appropriate proposals for developing further Community policy on the lead content of petrol. Thus it was foreseen in 1978, when the directive was agreed, that the levels could and should be changed if new knowledge justified it.

NIGEL HAIGH

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No contest?

SIR — *Nature* is highly valued by the scientific community not only for its scientific content, but also for its unwavering position on matters of scientific moral behaviour — and last but not least, the standing of its advertisments Therefore I would like to draw your attention to an instance which I consider as reprehensible practice, in which, in this case, *Nature* has been unwittingly the vehicle

In *Nature* Vol 285, 26 June 1980, the Malaysian government announced (page xxvii) a world-wide competition to find a speedy and accurate method of determining the dry rubber content (DRC) of latex. I sent an entry to this contest on 18 December 1980.

A letter of the Malaysian Rubber Research and Development Board (MRRDB), dated 3 March 1982 stated: " . . [we] regret the delay in the final judging; this has been due to the good response [63 entries, *note M.B.*] received and the need to test out some of the entries".

Without news for several months from the MRRDB, I wrote them a letter on 26 October 1982, from which I quote: "I presume this final judging has taken place. Sincere scientific curiosity, besides the sportsmanship of having participated, prompts me to ask the communication of the optimal method having been selected. Perhaps it could be published. . ."

From the answer of the MRRDB (10 November 1982): "Judging of the Dry Rubber Content determination competition took place on 28 October 1982 and regretfully, of the 63 entries received from all over the world, including the one entered by you, there has been no outright winner as none of the entries met the criteria of an ideal method for DRC determination."

Conclusion: for the price of an advertisment — and the vague promise of the sum of about $10,000, which is a mere trifle as research budgets go — the MRRDB did perhaps not obtain the dream-method, but it may be estimated that a significant fraction of the entries yielded enough valuable ideas, elements and propositions to obtain, through very little further expense, the ideal method, provided that one exists. Really, the MRRDB got quite cheaply a great deal of intellectual effort.

Had the advertisment in question appeared anywhere else than in *Nature*, I at least, and perhaps more of the 62 colleagues who sent entries would have perceived beforehand the unilaterality and the arbitrariness behind such a "contest".

MICHEL M. BENARIE

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Vitamin C reviewed

SIR — In reviewing our book "Vitamin C in Health and Disease" (*Nature* **299**, 760), Dr Rivers has had to contend with the problem of assessing three books at once and the self-inflicted difficulty of having apparently pre-judged the debate on vitamin C. Rivers's view appears to be that the vitamin's only function in man is to prevent the symptoms of overt clinical scurvy. He may be right, but as we emphasize this possibility in the concluding paragraph of our book, it is surprising that he accuses us of having an "atrophied critical faculty". However, he apparently draws this conclusion from a series of comments about the book which are simply not true, and it is these I wish to refute.

He does not exclude our book from his general observation that all three texts claim that vitamin C will not only cure scurvy but colds, cancer and cardiovascular disease. We make no such claim. We include in our evidence on vitamin C and the common cold the statement that randomized, blind, clinical trials have indicated that megadose vitamin C therapy has almost no effect on either the frequency or length of the cold. However, there is some evidence that there are decreases in the severity of the symptoms. For cardiovascular disease and cancer, we suggest that although vitamin C deficiency may be implicated in the aetiology of these conditions, only long-term, large-scale, randomized, controlled trials can confirm or refute such roles for the vitamin.

Rivers makes a more detailed criticism of one section of the book and again he is in error. In the figure he refers to, there is both a highly significant difference between the two groups and a significant correlation in one of the groups alone, a finding that he was unable to see by eye. To us, this indicates an association between hepatic vitamin C and rate of cholesterol transformation, without suggesting that a cause and effect relationship has been proven. The section which includes this graph makes no claim, as Rivers states, that changes in vitamin C status are central to fluctuation in mortality from ischaemic heart disease. Indeed, we warn that such evidence is only circumstantial and indirect.

In our book on vitamin C in health and disease we have attempted to summarize the available evidence. Dr Rivers may honestly feel that we have leant too far towards those who support a role for vitamin C in the prevention of a whole variety of diseases and he is entitled to his opinion, but he should have supported his views by statements which are correct.

C.J. SCHORAH

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Jungle law

SIR — J. Fleuris (*Nature* 18 November, p.212) is correct in stating that *orang* is Malay for man (though *person* is more accurate, as orang has no sense of gender), but *orang utan* has orang-utan only as a secondary meaning. Its literal meaning is man (or person) of the jungle (*utan* being the common word for jungle, as opposed to *ulu* which means up-river and has come to mean jungle by extension).

J.R. GOSDEN

Eskbank, Midlothian, UK

Chinese names

SIR,— M.C. Mckenna (*Nature* 18 November, p.212) oversimplifies when he states that Chinese given names are now hyphenated and the second syllable is not capitalized in English transliteration. While the use of the hyphen to link given names is widespread, it is by no means universal and the use of a capital for the second syllable is at least as common as lower-case.

Moreover it is now common in journals from Mainland China for the given names to be written as one word, for example Chen Yurong. This practice still enables the surname (usually one syllable) to be distinguished easily from the given names (usually two syllables) but the single-syllable given name (such as Pei, in Wang Pei) may still cause difficulty. It is not unknown, either, for "helpful" journals from Chinese and other ideographic language sources to present transliterated names in a Western order, without an obvious indication of their practice.

SUSAN JELLIS

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NEWS AND VIEWS

Oncogenes in solid tumours

One of the most obvious quibbles about the recently isolated clutch of human oncogenes should be quenched by the paper on page 539 of this week's issue. Since most of the oncogenes, including the first to be sequenced (see *Nature* 11 November, pp.143 and 149), have been derived from tumour cell lines cultured over many generations, the question has naturally been raised whether the supposed oncogenes might be an artefact of cell culture. But data now reported from the Laboratory of Cellular and Molecular Biology of the National Cancer Institute show that oncogenes can be found in cells taken from a variety of solid tumours surgically removed from human patients some time ago and since kept in a deep freeze. The point is important because it reinforces confidence in the pathological relevance of the discovery reported last month that cells cultured from a human bladder carcinoma contain an oncogene whose structure (described by R.A. Weinberg's group at the Massachusetts Institute of Technology and by Mariano Barbacid's group at the National Cancer Institute) differs from that of a normal human gene by a single substitution of one nucleotide by another. What now also emerges is good (if circumstantial) evidence for at least a second human oncogene. And oncogenes of some kind have turned up in several different types of human carcinoma — the lung (one), the pancreas (one), the colon (two) and an infantile sarcoma known as embryonal rhabdosarcoma.

The human oncogenes whose existence has been demonstrated in these solid tumours (and in a number of cell lines derived from a variety of other tumours) are, however, not the same as that reported last month by the Weinberg and Barbacid groups. They showed by sequencing nucleotide bases of an oncogene in a human bladder carcinoma that the oncogene differs from a naturally occurring normal gene only in a single base substitution, the effect of which is to substitute the amino acid valine for glycine in the twelfth position along the length of a protein molecule called p21. (The nondescript name is a sign of how little is known of the normal function of this material: "p" stands for "protein", "21" for the molecular weight, approximately 21,000). On this occasion, the nucleotide sequences of the oncogenes have not been determined directly, but have instead been inferred from the readiness with which cloned stretches of the DNA concerned will combine with the oncogenes of a variety of retroviruses.

The striking result is that most of the human oncogenes now found are related to the oncogenes carried by the retrovirus known as the Kirsten murine sarcoma virus. Indeed, in the malignant human cells, whether from tumours or by cell-culture, the version of the p21 protein produced is often antigenically similar to that produced in mouse cells transformed (or made malignant) by the Kirsten virus. From the known structure of the virus genes, the consequences for p21 should consist of another substitution for the glycine at position twelve. Nobody will be surprised if, in the human cells as in the virus, the glycine is this time replaced by serine, not valine. And given that the retrovirus known as the Harvey murine sarcoma virus is known to produce an altered p21 protein in which the same critical glycine is replaced by arginine, it will no doubt be only a matter of time before some human cancer associated with that mutation turns up. It is also, however, clear that mutations of the p21 gene are not a sufficient explanation of the malignant condition.

One of the intriguing features of this week's report by Barbacid's group is that cells from the two fresh colon carcinomas tested have oncogenes differing both from each other and from the Kirsten version of the p21 gene (which, nevertheless, turns up in a cell line derived from another colon carcinoma). It will be fascinating to see what are the nucleotide sequences in these genes, as it will be to know why the gene product of the single case of pancreatic carcinoma studied appears to have a molecular weight of 27,000, not 21,000. Meanwhile, Barbacid and his colleagues have taken advantage of the availability of normal as well as cancerous tissue from the patient with the pancreatic carcinoma to demonstrate that DNA from the normal tissues will not convert mouse cells of the NIH line known as 3T3 from their usual (nearly malignant) condition into cells that are frankly malignant. That has an obvious bearing on the question still to be settled of whether human oncogenes of the two types now characterized occur in apparently non-malignant cells, which in turn bears on the question whether in real cells (as distinct from those of the 3T3 cell line) the presence of an oncogene is a sufficient cause of cancer.

Already, however, it is clear that the two human oncogenes now defined are only a small part of this unfolding story. Thus the oncogenes known to be involved in tumours of the white blood cells, leukaemias, lymphomas and the like, but mostly in other than human animals, are quite different. Two important papers on that subject appeared last month in *Proc. natn. Acad. Sci. U.S.A.* (79, 6971 and 6999; 1982), even if their relationship with oncogenes is apparent only in the light of more recent information. Both papers are concerned with plasmacytomas, tumours of B lymphocytes secreting immunoglobulins. In the mouse, it is commonly observed that the end of a chromosome 15 has moved, in malignant lymphocytes, to the region of chromosome 12 where the gene for the heavy chain of immunoglobulin is located. Alternatively, a piece of chromosome 15 may finish up on chromosome 6, where the κ-chain genes are based. Jerry Adams and his colleagues in the Walter and Eliza Hall Institute of Medical Research have now cloned from a plasmacytoma a transcriptionally active segment of chromosome 12 which appears to have been derived from elsewhere, conceivably chromosome 15. And Kathryn Calame (from the University of California School of Medicine at Los Angeles) and her colleagues have identified in two plasmacytomas secreting immunoglobulin A the physical conjunction of a segment of chromosome 15 with part of the gene for the immunoglobulin A constant region gene of chromosomes 12 (to be precise, it is joined to that one of the two chromosomes 12 which is not actively involved in immunoglobulin A production).

From results reported from a recent meeting (see *Nature* 2 December, p.403), it now seems that these two groups — and Marcu *et al.* before them (*Proc. natn. Acad. Sci. U.S.A.* 79, 6622; 1982) — have been observing the translocation of the oncogene known as c-*myc* from chromosome 15 to an active region of chromosome 12 or, alternatively, the transfer of this active region to the site of c-*myc* on chromosome 15. Either way the proposal is that the c-*myc* gene thereby becomes expressed and that this is associated with the oncogenetic process.

As a service to readers, the staff of *Nature* will attempt at intervals in the weeks ahead to draw attention to papers appearing elsewhere which are judged to be important contributions to the quickly developing oncogene story. The assistance of readers and of the authors of papers published elsewhere would be appreciated. Communications (by letter or telephone) should be addressed to Dr Peter Newmark in the London office.

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Safe with Cepheids?

from David A. Hanes

MEASUREMENTS of the period of oscillation of the pulsating supergiant Cepheid variable stars have underpinned our estimates of the cosmic distance scale and thus of the age of the Universe for more than half a century. If a study[1] recently published by M.J. Stift and first brought to the attention of astronomers at the General Assembly of the International Astronomical Union in August is correct, however, this application will have led to serious errors.

By astrophysical good fortune, Cepheids combine several properties which make them potentially excellent indicators of distance: they are intrinsically luminous supergiants, and so can be seen even in external galaxies; their luminosity varies with considerable amplititude, so that they draw attention to themselves in crowded star fields; and they vary with well defined periods which are proportional to their intrinsic luminosities. Thus a simple determination of the period of an individual Cepheid allows the intrinsic luminosity to be calculated — comparison with the observed mean apparent luminosity of the star then yields a direct distance estimate; provided that the period–luminosity (PL) law has been correctly calibrated with reference to Cepheids whose distances are independently known.

The importance of the PL law was recognized shortly after its discovery[2] in 1912 in a sample of Cepheids at a common distance in the Large Magellanic Cloud, the nearest galaxy outside our own Milky Way. The subsequent identification[3] of Cepheid variables in the Andromeda nebula (M31) and the Triangulum nebula (M33) resolved the controversy over the nature of the spiral nebulae, proving that our Galaxy is not unique, and set the stage for the discovery[4] of the expansion of the Universe. The succeeding half century has seen many revisions[5,6] of the extragalactic distance scale and inferred age of the Universe, but that scale still depends in large measure on the distances implied by the Cepheid variables (see *News & Views* **299**, 297; 1982).

It is in the calibration itself that difficulties arise. Cepheids are young massive stars in short-lived late stages of stellar evolution; thus they are rare. None is close enough to permit a straightforward measurement of distance (say, by parallactic triangulation) and the calibration relies largely on the identification of a handful of Cepheids in clusters or associations whose distances can be derived by other often less precise means. Moreover, Cepheids are found preferentially in the plane of the Galaxy, and may be considerably obscured and reddened by interstellar dust. This problem is exacerbated by the youth of the Cepheids: they may be found still embedded in the locally dust-rich environments from which they formed. Thus it is difficult to assess with what precision we know the absolute luminosities and colours of the calibrating Cepheids.

In external galaxies, further problems arise. There is some evidence[7] that the PL law differs from galaxy to galaxy, perhaps as a consequence of different heavy-element abundances. Measurements of stellar brightness are difficult in crowded fields near the limits of big telescopes. Perhaps most important, only the extremely luminous Cepheids (those of the longest periods) can be detected in remote galaxies; such stars are not found among the calibrating sample, and extrapolations of the locally calibrated PL law are required. A clear understanding of the law is therefore essential.

What form does it take? It has been argued[8] that a simple PL law is an incomplete description of the phenomenon. With age, evolving stars change their radial distributions of mass, composition, rate and source of energy generation, temperature and opacity. At certain critical phases, manifested observationally by characteristic intrinsic luminosities and surface colours (or, equivalently, surface temperatures), stars may become unstable to pulsational vibrations. The region of the luminosity–colour plane within which such variations occur is referred to as the 'instability strip'. Elementary physical considerations suggest the necessity for a three-argument period–luminosity–colour (PLC) law — at a given period Cepheids may exhibit a range of mean luminosities, with the intrinsically brighter at that period being the hotter (bluer) stars, while their fainter counterparts are cooler (redder) — or vice versa. No such relation will arise if the instability strip is very narrow, or if some compensating structural difference fortuitously cancels the dependence on colour. An analysis of available data did, however, originally seem to support the three-parameter PLC formulation — although that analysis[8] has subsequently been hotly criticized[9,10].

Supporters of the full PLC law argue that its use improves the Cepheid distance estimates to the fundamental limit permitted by the measuring uncertainties of the observational data themselves. However, there are difficulties. It is disquieting that the colour coefficient ($\beta = 2.7$) derived[8,11] for the PLC relation so nearly matches the effect ($\beta \sim 3$) which would be produced by a component of interstellar obscuration; that is, for Cepheids of given period the fainter (obscured?) stars are redder by almost exactly the amount that would be produced by normal interstellar dust giving rise to the observed dimming. While this may be mere coincidence, it suggests that differential obscuration may be largely responsible for the apparent PLC law, in which case the subsequent technique of determining distance may be erroneous, especially as it depends in certain applications upon lengthy extrapolations of the law.

The recognition of this problem recently sparked two separate critical assessments, by J. Brodie and B. Madore[12] in Cambridge and by V. Clube and J. Dawe[10] in Edinburgh. The Cambridge study was based on Monte Carlo simulations of randomly generated data sets, obeying underlying perfect PLC laws but variously affected by reasonable random errors of photometry and by plausible randomly assigned amounts of obscuration. The conclusion was that the coefficient could be in excess of $\beta = 5$, but that in the presence of moderate amounts of differential obscuration the intrinsic PLC relation is completely masked by the dominant extrinsic trends. On the other hand, the Edinburgh study, which criticized (*inter alia*) the original[8] semitheoretical determination of the colour term, concluded that near-zero or negative values were favoured.

M. Feast and L. Balona published a swift rebuttal[13] of the Cambridge study, with the distinction between the analyses being one of mathematical methodology: in the rebuttal, maximum likelihood techniques of arguably greater reliability were shown to reconfirm the original PLC derivation. It is this conclusion that is now under spirited attack by M.J. Stift[1]. Like Brodie and Madore, he uses extensive numerical simulations and introduces all conceivably relevant effects: the photometric measuring errors; the effects of obscuring material, randomly assigned; the presumed true physical width of the instability strip; a spread in Cepheid properties caused by

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mass loss among a sample of originally identical stars; and so forth. His analyses show that the maximum likelihood method systematically overestimates β, whether the data are affected by correlated (obscuration-induced) or uncorrelated errors. Indeed, Stift concludes that it is impossible in such analyses to distinguish between $\beta = 0$ — the irrelevance of a colour term — and $\beta = 2.7$, the generally accepted value: the maximum likelihood estimate is indeterminate. His own analyses merely suggest that β must lie in the broad range 0-3. More importantly, he concludes that the inclusion of the colour term is irrelevant and generally erroneous in the use of Cepheids; he recommends the complete abandonment of the PLC relationship as an indicator of cosmic distance.

Such a conclusion is particularly damaging for the Sandage–Tammann construction[6,14] of the universal distance scale, which is already under critical attack[9,15,16] for other reasons, because their entire scale is predicated on the use of the PLC relationship for Cepheids in nearby galaxies — the first stepping-stones in a hierarchical construction. The present study is more supportive of an alternative treatment[17] of the Cepheid variables by de Vaucouleurs, which, when coupled with other indicators, yields a cosmic distance scale half that proposed by Sandage and Tammann. Thus Stift's provocative results may help (if correct in all particulars) to resolve this outstanding controversy, a subject of often heated and occasionally acrimonious debate for the last decade. Observational cosmologists are eagerly awaiting the next instalment. ☐

Microbial galley slaves

from Ralph A. Lewin

SOME of the weirdest and most wonderful microorganisms are to be found in termite guts, veritable zoos of strange protozoa, and among these a devescovinid flagellate is especially notable for having enslaved bacteria to move it around. Some recent papers by Tamm[1,2] provide revelations on this subject, trumping science fiction with science facts.

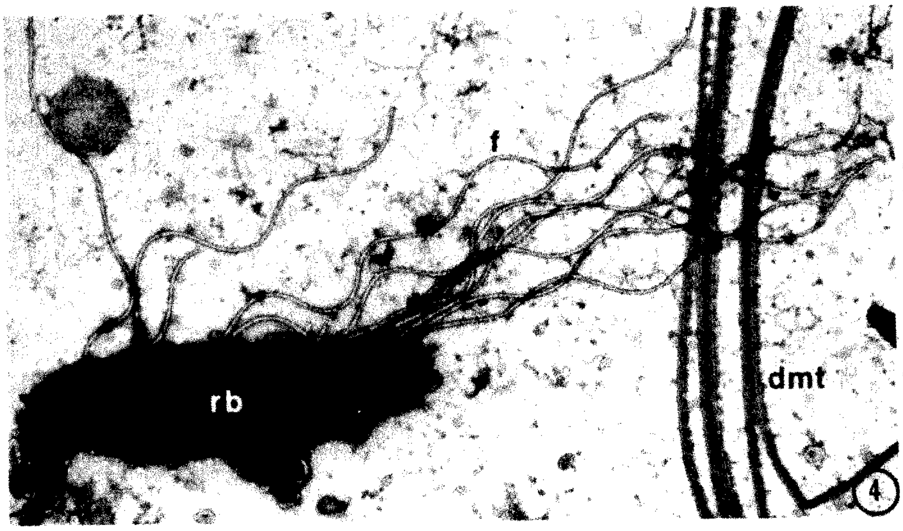
Devescovinids are fairly large, as microbes go; their cells are 100–150 μm long and 30 μm wide. They occur in the digestive systems of certain Florida termites (*Cryptotermes cavifrons*), where they slither around in continuous motion. Each cell has a pointed front end tipped with a sort of nose-cone or conning-turret, which is constantly rotating at about 30 r.p.m. (Look at it face-on, and you see that it always turns clockwise. How a single lump of protoplasm can have a swivel like this is one of its mysteries; it must have no microtubules in its 'neck' regions, or they'd soon get screwed up.) At its front end the animal has four whip-like cilia, about as long as the cell and some 400 nm wide, with the usual arrangement of 9 + 2 microtubules found in other eukaryotes. Three of them lash to and fro, forwards and back, while the fourth trails backwards like a sort of rudder. However, the three beating cilia are not the main mechanism of locomotion for this (as yet unnamed) devescovinid, since most of the work is apparently accomplished by some two or three thousand rod-shaped bacteria, 2–3 μm long, attached to its surface. These little galley slaves lie end to end in more than a hundred single files, firmly embedded in helical grooves. On its outer side each bacterium bears a dozen flagella. These are 4–5 μm long but only 18 nm wide, and are composed of longitudinal rows of flagellin molecules like those of, say, *Escherichia coli* (though in *E. coli* they are borne on all sides of the cell). Some of the flagella from each bacterial cell trail backwards and join those of the cell behind to form a helical bundle about 24 flagella thick. Hydromechanical forces are evidently responsible for aligning and synchronizing their movements. As the individual flagella rotate, so do the helical bundles, and this is what propels the protozoan on its way. (Other ectosymbiotic bacteria — longer, thinner and without flagella — are attached along the ridges between the grooves; their role is so far completely unknown.)

A devescovinid slithers along at a rate of about 100 μm s^{-1}. As large protozoa go — *Paramecium*, for instance — that's not very fast, but since the motors are basically bacterial it isn't a bad speed at all. The protozoan vessel is propelled three or four times as fast as a free-swimming *E. coli* cell, even though its flagella are of similar size and rotate at much the same speed (50–150 Hz). Tamm attributes their celerity to the fact that devescovinids glide over solid substrates — probably bits of wood ingested by the termites — and that less energy is dissipated at a phase interface than free in a fluid.

Can one be sure that it is really these bacteria that are responsible for the motility of the protozoan? Two lines of evidence, at least, indicate that this is so. First, many fragments of protozoan cells — crushed, for example, under a coverslip — rotate actively if they bear bacteria, although they may completely lack any of the four devescovinid cilia: the more bacteria they bear, the faster they spin. Bacteria liberated from crushed host cells can swim, too. Second, a proton ionophore such as dinitrophenol (10 mM), which interferes with the motility of bacterial flagella, reversibly arrests the movement of the devescovinid though not the beating of its own cilia. On the other hand, certain polyene antibiotics, toxic to most eukaryotic cells, inactivate the four cilia of the protozoan but have little effect on its gliding activity. (A specific inhibitor of the ATP mechanism of protozoan cilia would presumably not interfere with the gliding movements of the devescovinids, but Tamm did not mention having tried this.)

How and why could such a system have evolved? We might start by asking what benefit the protozoa could derive from being able to move at all. Perhaps such slithering around enables them to keep making new contacts with the bits of wood on which they are presumed to feed. (Many symbiotic protozoa digest lignocellulose

Electron micrograph of a rod bacterium (rb) isolated from the surface of a devescovinid. The flagella arise from what would be the exposed surface of the bacterium and are shown in comparison with the douoblet microtubules (dmt) from the devescovinid's own flagellum.



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with the aid of hyper-symbiotic intracellular bacteria, but that's another story.) Perhaps motility, with some kind of orientation mechanism located in the conning-tower apparatus at the pointed end, helps to ensure that the devescovinids stay cozily ensconsed inside the termite's gut and are not excreted or shed when their insect host casts its skin. Maybe the general stirring of the intestinal contents helps to keep gut fermentations operating effectively. But why don't these protozoa depend entirely on their own ciliary apparatus, and 'go it alone' as do most other motile microbes? Are the three beating cilia too weak to move such relatively large cells around? Apparently so; or perhaps it is cheaper, energetically, to enslave or enlist bacteria to do the rowing than to row oneself. However, as any trireme skipper knows, even slaves have to be fed if they are to continue to man oars effectively. Presumably some nutrients are transferred through the cell membranes of the protozoan grooves to keep the hard-working bacteria going, but so far this subject seems not to have been investigated.

As for the steps by which such a system of microbial enslavement might have evolved, we can only hazard a guess. Many animals are covered with bacteria; in some invertebrates they seem to be highly specific. Little flagellate cells may shift bigger things around, sometimes fortuitously (witness the rotating eggs of seaweeds such as *Fucus*, spun by the crowds of spermatozoids attracted to their surfaces) and sometimes in an apparently purposeful fashion (as in the green alga *Prasiola*, where the fertilized egg gains the ability to swim by adopting one of the flagella of its successful male partner[3]). As Tamm reminds us, there is another genus of protozoa related to the devescovinids, *Myxotricha*, found in the gut of another kind of termite, from Australia, which has rows of bracket-like processes along its sides, each bracket bearing a motile spirochaete; by means of these symbiotic cells, it can swim in much the same way as the devescovinid described above, although at only about one-fifth of the speed. This remarkable symbiotic system, in *Myxotricha*, has been known and studied for about 20 years, but Tamm's observations on devescovinids are much more recent and quite as fascinating. ☐

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Bird coloration and parasites — a task for the future?

from Paul H. Harvey and Linda Partridge

NATURAL selection for defence against parasites may have more significance for evolutionary biology than has often been supposed. Increasing awareness of host-parasite interactions has led to suggestions that they have a key role in population regulation[1,2], in the maintenance of genetic polymorphism[3], in social organization[4,5] and even in the evolution of sex[6–8]. Now, W.D. Hamilton and Marlene Zuk of the University of Michigan have come up with the hypothesis[9] that parasite resistance is even important in the evolution of bird coloration. The idea is that birds choose mates with bright plumage and complex songs because these characters are correlated with heritable resistance to parasites.

For a character to be heritable genetic variation must exist. Ever since Haldane[10], population geneticists have known that defence against different parasites could lead to the maintenance of such variation in a host species. Most models of the process assume that increased protection against one type of parasite leads to increased susceptibility to another; this has been verified in several cases (see ref. 3), though mainly among plants and their parasites. However, not all models make this assumption. For example, if defence mechanisms against parasites are costly in terms of potential reproductive success, then when parasites are common genes for defence may be favoured, but when parasites are rare those same genes may be at a disadvantage[3].

Genetic variability in the host species can be maintained without there being a single equilibrium point: limit cycles and other patterns of temporally changing gene frequencies are features of some models. If cycles are long, then genes for parasite resistance which are favoured by natural selection in one generation are, on average, those likely to be favoured in the next generation. As a consequence, when cycles are long, genes for choosing mates that have high parasite resistance will be favoured by sexual selection.

But how can animals recognize that potential mates possess the correct genes? One way would be to assess their parasite loads directly: when an animal has few parasites, it means it has high resistance. A more indirect measure might be via plumage characteristics. That, at least, is the suggestion made in the recent paper by Hamilton and Zuk[9]: "The methods used should have much in common with those of a physician checking eligibility for life insurance . . . the choosing animal should unclothe the subject, weigh, listen, observe vital capacity, and take blood, urine and faecal samples". Although they cite no relevant evidence, they go on to argue that "general good health and freedom from parasites are often strikingly indicated in plumage and fur, particularly when these are bright rather than dull or cryptic". Within bird species, they predict that the most brightly coloured animals should have low parasite loads, and, *ceterus paribus*, be favoured mates. In contrast, across species, bright coloration should be found associated with high species-specific parasite loads — species subject to a wider variety of parasites will have experienced stronger selection. They were only able to test the latter prediction, although the former is probably the more telling.

Data on four or five haematazoa and on microfilarial worm infestations of passerine birds from five sites were extracted from the literature. Both sexes of 109 different species were scored for degree of 'brightness', and in those same species the songs of the males were scored for 'complexity'. Male brightness (1), female brightness (2), male song (3), male showiness (1 + 3) and bisexual showiness (1 + 2 + 3) show weak but highly significant increases with recorded parasite load across species. The one exception is negative associations of brightness, song and showiness with the generic 'disease' *Toxoplasma*. A footnote to the paper points out that, although these same general patterns of increased brightness, song and showiness with parasite load are found in grouse, the opposite correlations hold for owls.

Hamilton and Zuk conclude that the results conform to their model, although "evaluation of the many plausible and overlapping theories about display and sexual selection, including our new one, is a daunting task and must be left to the future". That is true, but it is unfortunate that their report was not presented at a greater length. Then they could have presented more than summary statistics of their data and they could have considered other correlates of bright coloration among bird species. Two were mentioned and considered to be in agreement with their theory: bisexual brightness and sexual dimorphism are lower on islands[11] (there are fewer parasites on islands), and larger-bodied species tend to be more brightly coloured[12] (larger birds provide a bigger target for vectors, and can tolerate more insect bites). But what of the other correlates pointed out by Baker and Parker[13] in their detailed study of coloration in 516 species of Western Palaearctic birds? For example, diurnally active birds are more conspicuous than nocturnally active birds, gregarious birds are more conspicuous than solitary birds and concealed nesters

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are more conspicuous than exposed nesters. Baker and Parker conclude that "in every case the more colourful category is likely to be a less profitable prey to a predator than the more cryptic category" and "that it is possible to explain variation in the coloration of adult birds largely in terms of the selective pressures generated through predation risk". We can also make *post hoc* explanations for why each of those same categories should be subject to higher parasite infestations.

The time has come to make predictions which will enable us to distinguish between these and alternative hypotheses. Surely, after taking possible taxonomic effects into account, different hypotheses should make different predictions about other ecological, behavioural or morphological correlates of bright coloration? □

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Vaccination programmes and herd immunity

from Robert M. May

VIEWED from the level of the population as a whole, immunization programmes have two primary effects.

The more obvious, or 'direct', effect of vaccinating some fraction of a population against a particular infection is that most of the vaccinated individuals are thereby protected. The ideal vaccine or toxoid is one which produces immunity in all treated individuals (100 per cent efficacy), and where this immunity is lasting. Current measles vaccines, for instance, approach this ideal, apparently inducing lifelong immunity with about 95 per cent efficacy.

Less obviously, the immunization of some fraction of the population is likely to have the 'indirect' effect of diminishing the 'force of infection', defined as the probability that an unvaccinated individual will contract the infection within a specified time interval. If fewer people are candidates to run around coughing or sneezing at you, your chances of acquiring infection are likely to be smaller. One consequence of the existence of such indirect effects is that it is not necessary to immunize everyone in order to eradicate an infectious disease; once a sufficiently large fraction of the population has been protected, the associated indirect effects can drive the reproductive rate of the infection — the average number of secondary cases produced by an infected individual — below unity, so that the infection cannot be maintained[1,2] (except by immigration). It is the intuitive appreciation of these facts that lead many people to decide their best vaccination programme is one where almost everyone else is vaccinated while they are not, so that they are indirectly protected without incurring any of the risks or inconveniences associated with direct protection. Such dissonance between the interests of the individual and the interests of the group arises, of course, in many areas of evolutionary biology and elsewhere. In what follows, we shall meet some subtle variations on this theme.

Bearing in mind both direct and indirect effects, we may ask what happens to the population as a whole — to 'herd immunity' — as a result of immunizing, say, 50 per cent of the population.

One answer comes from the extensive mathematical literature dealing with the transmission and maintenance of infectious diseases. Essentially all such theoretical work is based on the assumption of 'homogeneous mixing', whereby new infections appear at a rate directly proportional both to the number of infectious individuals and to the number of susceptibles. That is to say, school and family associations, and all other kinds of social groupings, are averaged out; infections result from binary encounters within a homogenized population. Under this assumption, implementing a vaccination programme that is not sufficiently intense to eradicate an infection will lead to a new steady state in which the number of susceptible individuals is exactly the same as in the unvaccinated population[3]. At first sight, this result may seem surprising; intuition suggests the proportion susceptible should decrease as vaccination coverage increases.

The theoretical result is, however, easily proved. First, let R_0 denote the basic reproductive rate of the infection, the average number of secondary cases produced when an infected individual is introduced into a wholly susceptible population[4-6]; R_0 depends on the basic biology of the infectious agent, and on all manner of social and environmental factors that affect transmission. Next, suppose that only a fraction x of the population is susceptible, the other individuals having acquired immunity either by vaccination or by recovery from natural infection. In a homogeneously mixed population, the average number of secondary infections produced by each infectious individual is then $R = R_0x$. But at equilibrium each infection must produce on average exactly one secondary infection, $R = 1$. Hence, for an infection that is established at some endemic steady state, the fraction susceptible is $1/R_0$. The answer depends only on R_0, and is independent of the proportion vaccinated.

To make the result a bit more concrete, consider the example of an infection with $R_0 = 10$ (which is very roughly the case for many childhood infections in Western countries). The equilibrium state before any vaccination has 10 per cent susceptible, with essentially all the remaining 90 per cent having acquired immunity following recovery from infection (the typical infection runs a relatively short course, so the remaining fraction that are actually infectious at any one time is very small although, of course, crucial for maintenance of the infection). Suppose now that we move to a new steady state in which 50 per cent of the population are immunized by a vaccine or toxoid. If there were no indirect herd effects of immunization, the remaining 50 per cent of the population would be divided between susceptibility and acquired immunity in the same proportions as in the original population, to give a total of 5 per cent susceptible and 95 per cent immune (50 per cent from the control programme, 45 per cent following natural infection). The existence of indirect effects, however, leads under the assumption of homogeneous mixing to there still being 10 per cent ($1/R_0$) susceptible and 90 per cent immune (50 per cent from the control programme, 40 per cent following natural infection). By the same token, immunization of more than 90 per cent of the population (or, more generally, more than a fraction $1-1/R_0$) leads to eradication of the infection, by virtue of the indirect effects[7]. In the absence of such indirect effects, almost 100 per cent immunization would be required to eradicate infection from a very large population.

Many people have observed that real populations are rarely, if ever, 'homogeneously mixed', with susceptibles and infecteds bouncing around like the molecules of an ideal gas. The assumption may nonetheless give a good description of what happens on the average; as Bartlett[8] has written: 'even if a multiplicity of detailed causes is operating to produce the observed broad classes of events, it is often

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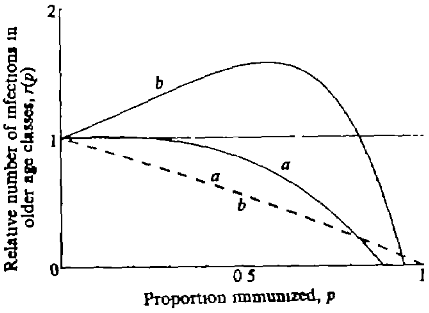
an economy of thought in the sense of Mach to ignore these and appeal merely to the operations of chance and the laws of averages'. This issue cannot be resolved in the abstract. What we need are good empirical tests of the validity of the predictions made by the simple theory.

One especially interesting such test has recently been made for measles by Fine and Clarkson[9]. Analysing the data that are available for age-specific incidence and immunity levels for measles in England and Wales since 1950, they find the 'total number of individuals susceptible to measles has remained relatively constant', at around 4 to 4.5 million. This corresponds to about 9 per cent of the population being susceptible, implying R_0 is around 11–12; this accords with other independent estimates[10]. As Fine and Clarkson emphasize, there are biases and deficiencies in the available data (having to do mainly with the notification of cases), and methods of correcting for such biases depend to a degree on the assumption of homogeneous mixing. Thus their test of this assumption is not altogether free of some circularity. I think, however, that Fine and Clarkson's analysis is important, showing that homogeneous mixing represents a useful working approximation for measles in England and Wales, and therefore possibly for broadly similar infections elsewhere.

Weighing risks

In practice, immunization programmes must take account of additional complications arising from age-specific aspects of the vaccination schedules and of the infection processes. If the bad effects of infection are the same at all ages, then any vaccination programme (no matter how low the coverage) will reduce suffering to some extent, provided only that the vaccine is safe. In this situation, it is in principle a relatively simple matter to weigh the health benefits of a vaccination programme against the risks of vaccination itself (although an economic cost–benefit analysis is likely to be more complex, involving the familiar difficulties of assigning monetary values to physical disabilities). The current controversy over vaccination against pertussis (whooping cough) is a case in point. Here the debate is clouded by uncertainties about the risks of vaccination, by the possibility that immunization protects against the symptoms of pertussis but not against becoming infected and infectious (so that no indirect benefits are realized by vaccination) and by a certain amount of emotionalism. I shall not deal further with this vexed subject, lest this article get out of hand.

Such calculations become trickier when a disease has been eradicated from a particular region or country, so that incidence of the disease depends on chance episodes of importation. Becker[11] has shown how to estimate the number of fatalities to be expected from such an episode in an unvaccinated population, before quarantine and vaccination get things under control, and how to weigh this against the number of fatalities to be expected in association with a vaccination programme. This general kind of calculation underlay the UK and US decisions to discontinue routine vaccination against smallpox in 1971: in the UK, the years 1951–1970 saw around 100 deaths from smallpox vaccinations (roughly one death per million primary vaccinations) and approximately 37 from smallpox itself[1].



The number of infections among females in older age groups (specifically, between 18 and 35 years) when a proportion p of the females are protected by vaccination; the number of infections are calculated as a ratio, $r(p)$, to the corresponding number without any vaccination. The solid curves are for a vaccination policy in which a proportion p of both males and females are immunized at age 2, and the dashed curves are for a policy in which only females are immunized at age 12. The solid and dashed curves a are for $R_0 = 7.5$, and the curves b are for $R_0 = 13$.

Age-dependent effects

For many viral infections, the chance of serious complications arising is greater if the infection is acquired at an older age. For example, although the probability of encephalitis occurring as a complication of measles is low, this probability rises roughly linearly with age at infection[12] (at least through the first 20 years of life). In such circumstances, it is important to note that indirect effects of immunization or other factors that diminish the 'force of infection' within a population will correspondingly increase the average age at which infection is acquired. There is evidence to this effect from vaccination programmes against measles and rubella (German measles)[9,12].

In short, under a vaccination programme stopping short of eradication, fewer individuals will get sick, but those who do will do so on average at an older age. If fatalities or other complications are more likely at older ages, it is thus conceivable that certain vaccination programmes could actually increase the incidence of serious cases. The likelihood of such a perverse outcome is not easily guessed, but rather requires a close analysis of the detailed interplay among several factors: the way serious illness depends on age, the details of the vaccination programme and the basic biology of the transmission process (summarized by R_0).

It is probable that the history of poliomyelitis represents a 'natural experiment' of this general kind. The argument runs that, in earlier times, most people contracted this infection in the first few years of life, when it very rarely has serious consequences. Increasingly high standards of cleanliness in Western societies in the middle decades of this century led to lower values of R_0, and thence to a diminished force of infection and a consequent rise in the average age at which infection was acquired (albeit by fewer people, in total). Since paralysis and other complications are apparently much more likely for infections in teenage or older individuals, poliomyelitis paradoxically became more troublesome as its transmissibility declined. More recently, in a notable success story, the advent of safe, effective and cheap vaccines has effectively eradicated poliomyelitis in Western countries.

Rubella is a particularly striking example of an infection whose seriousness depends on the age at infection. Usually a mild infection, rubella can cause the damaging congenital rubella syndrome (CRS) in the offspring of women who acquire the infection in the first trimester of pregnancy. In a seminal paper, Knox[11] has shown how the incidence of CRS may indeed increase under some vaccination programmes.

The solid curves in the figure (which represents a slightly modified version[12] of Knox's simpler analysis) show the number of individuals who are infected between the ages of 18 and 35 — the main childbearing years in Western countries — when a proportion p of the total population (boys and girls) are successfully immunized at age 2, as a ratio to the number of individuals acquiring infection in this age range before vaccination was introduced. This ratio, $r(p)$, provides a rough estimate of the relative incidence of CRS to be expected for different values of p; more accurate calculations integrate over the product of the reported age-specific fecundity times the age-specific incidence of infection[12,13]. Curve a is for an infection with $R_0 = 7.5$ (roughly corresponding to rubella in the US and UK), and curve b is for $R_0 = 13$. In both cases it is assumed that the population is homogeneously mixed, and that everyone lives to age 75 and then obligingly dies (which is a reasonable approximation to the real survivorship function). From these solid curves a and b, we see that the incidence of infection among older individuals can increase under a vaccination programme with moderate levels of coverage (p not too large), and that this propensity becomes more pronounced as R_0 increases.

The dashed curves a and b (which are effectively indistinguishable) correspond to the solid curves a and b respectively, with the one difference that now a proportion p of girls, and only girls, are immunized at age 12. For given p, the two immunization policies provide the same amount of direct protection against CRS. The 'girls at age 12' policy has little effect on the overall force of infection within the population, and thus produces little in the way of indirect protection; hence the ratio $r(p)$ is roughly just $1-p$. The 'everyone at age 2' policy, on the other hand, does substantially affect the force of infection, producing indirect effects that both increase the average age at infection and eventually, for large p, lead to eradication. Hence, as shown in the figure, the former policy is better at low p and the latter at large p. For rubella, the cross-over point is for p in the range 75–85 per cent[12-15].

The solid curve, or 'everyone at age 2' vaccination policy against rubella, is essentially that followed in the US where immunization levels are in the 90 per cent range or above. Conversely, the dashed curve, or 'girls at age 12' vaccination policy, is essentially that adopted in the UK, where p is currently around 70–80 per cent. Each country thus appears to have chosen the correct policy for its particular level of vaccination coverage[16].

This analysis of vaccination strategies against CRS can be elaborated and made more realistic in many ways. In particular, Dietz[14] and Hethcote[15] have included the costs of the different policies in more exhaustive studies.

Most mathematical work on herd immunity and the effects of vaccination concentrates on comparing the initial and final steady states. The dynamics of the transition process, however, can be of great significance, expecially as programmes often undergo changes in midstream. Analysis of the age-specific dynamical effects is made complicated by the existence of several characteristic time scales, ranging from around 10 days or so for infection to run its course in an individual, to the 70 years or so that characterize the lifespan and turnover in a population at equilibrium. In some of the large and avowedly realistic computer models for these dynamical phenomena, apparently harmless approximations (such as updating the force of infection once a year instead of every few days) have led to spurious results because the intricacies associated with the different time scales are not fully understood. I think elaborate numerical studies can only be trusted if they are grounded on a clear understanding of the basic dynamical processes.

On the other hand, the most mathematically impeccable studies of the dynamics of vaccination programmes are largely fruitless unless tested against factual evidence. There is very little such work, and a clear need for more studies of the kind undertaken by Fine and Clarkson.

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Very high-energy gamma-ray astronomy

from P. V. Ramana Murthy and Trevor Weekes

THE high-energy region from 10^{11} to 10^{14} eV is the last window in the electromagnetic spectrum to be exploited by ground-based astronomers and one of particular relevance to high-energy astrophysics. Practitioners in the field met at a recent workshop* in Ootacamund, India to discuss their results: although there have been experiments in four continents over two and a half decades, the techniques used have been somewhat different and there has not always been agreement on the results obtained.

The flux of photons with energies above 10^{11} eV is very small so that detection by satellite, even with larger detectors than at present, would be extremely difficult, if not impossible. At energies greater than 10^{14}eV photons cannot be observed as they undergo photon–photon interactions with the universal 3° black body radiation and are absorbed over distances greater than 10 kiloparsecs. Fortunately for the ground-based astronomers, though, photons in the 10^{11}–10^{14} eV range are sufficiently energetic that when they interact with the upper atmosphere they produce an electromagnetic cascade that can be detected by its atmospheric Cerenkov radiation using simple light collectors. The great advantage of this technique is that the effective area for detecting γ rays is a few orders of magnitude larger than the geometrical area of the detectors themselves. Although attempts have been made to detect cosmic γ-ray sources using this technique since 1958, it is only in the past decade that sources have been detected.

Cygnus X-3, a binary X-ray source, first came into prominence when it underwent a massive radio outburst in 1972. Within a few days, γ rays of 10^{12} eV were detected from the source. Every year since then the flux, although variable, has been detected (Stepanian, Crimean Astrophysical Observatory). The emission shows two modulations, with 4.8 hour and 34.1 day periodicity, which also occur at X-ray wavelengths. The 4.8 hour light curve has been seen at energies $\gtrsim 10^{12}$eV for some time and is quite different from that seen at lower energies; the 34.1 day modulation has only recently been detected (Turver, Durham University) and appears to be out of phase with the X-ray variation.

Since high-energy γ-ray emission has been detected from the young pulsars in Vela and the Crab, it has been suggested that the Cygnus X-3 system may contain a young pulsar. A search for pulsations in the high-energy γ-ray signal in the range 4–40 ms has so far been unsuccessful (Porter, University College, Dublin).

An alternative model based on an analogy with SS433, a source with known jets of relativistic material, requires a black hole or neutron star to be in orbit with a helium star; the rotation axis of the compact object makes an angle with the plane of the system (Grindlay, Harvard-Smithsonian Center for Astrophysics). The analogy is attractive since SS433 is the most powerful source in the Galaxy and Cygnus X-3 may be the most powerful source yet detected.

There is no real problem in producing high-energy γ rays in pulsars; the difficulty arises in getting them away from the pulsar without absorption. Variability in the pulsed γ-ray emission is evidence for this difficulty and suggests that the pulsars that are regular emitters at X-ray and optical wavelengths may be quite different at these extreme energies. Previous reports of transient emission of 15-min duration from the Crab pulsar have been extended by the observation of 1-min variability in the pulsed signal from the Crab pulsar and 15-min variability from the Vela pulsar (Vishwanath, Gupta, Tata Institute of Fundamental Research).

The atmospheric Cerenkov technique finds application in another area — study

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*The 'International Workshop on Very High-Energy Gamma Ray Astronomy' was held at Ootacamund, India on 20–25 September 1982 and was jointly sponsored by the Smithsonian Institution, USA and the Tata Institute of Fundamental Research, Bombay, India

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of extensive air showers produced by ultra-high-energy cosmic rays. By studying air Cerenkov pulse profiles and amplitudes along with other observations, it is becoming possible to understand the charge composition of primary cosmic rays at energies greater than 10^{15} eV (Rao, Tata Institute; Tanahash, University of Tokyo; Clay, University of Adelaide; Turver).

Further progress in this discipline requires the development of new and more sensitive detection techniques. Improvement in angular resolution will lead to improved minimum flux sensitivities; such improvement may come from the imaging systems being developed independently at the Crimean and Whipple Observatories. Since the sources so far detected include an X-ray binary, pulsars and a radio galaxy, a small improvement in sensitivity could lead to a dramatic increase in the number of detected sources (Gursky, Naval Research Laboratory). With the development in this decade of more sensitive detectors at satellite γ-ray energies and of new experiments in neutrino astronomy, the role of the atmospheric Cerenkov technique in covering this window in the electromagnetic spectrum will become all the more important. □

Greek fireworks

from M.H. Day

THE Third European Congress of Anthropology was held in Greece at Petralona, near Thessalonika, at the end of September. Two main themes — the pre-*sapiens* stage of human evolution and the biology of migrating populations — attracted great interest and over 200 delegates from around thirty countries took part. One individual dominated the whole gathering, however, and that was Petralona man himself; unfortunately he was not present at the Congress since his fate was then still in the hands of the Greek courts of law. Until the courts decide whether to accede to Aris Poulianos's request that the skull be transferred to his museum at the Petralona cave, the Petralona skull remains aloof and incarcerated in its present resting place at the University of Thessalonika museum.

The first two days of the meeting were held at the Petralona site itself, in the splendid new facilities that have been provided by the energy and dedication of Poulianos, including a new tarmac road, a conference hall, a museum and laboratories. The Petralona cave nearby must be one of the most beautiful caves in Europe with hundreds of metres of vaulted galleries with stalactites and stalagmites; the cave is well illuminated and now engineered with walkways. Delegates wandered in and out of the cave freely and were shown anything that they wished to see.

The problems and the scientific disputes over Petralona man are of long standing and now rest principally upon the dating. Indeed, the conference was preceded by a brisk exchange of views on this subject in the pages of this journal[1-5]. The skull itself, billed as 'The Oldest Europaeoid' has features that make it crucial to establish its evolutionary position.

So muddy have the waters become that almost everything ever stated about the find and the site has been questioned, yet there are some facts that seem to have a solid consensus of support. First, there seems no doubt that at Petralona in 1960 a virtually complete fossil skull was found adherent to the cave wall. Second, the cave is a Pleistocene cave containing an extinct fauna. Third, it does have a stratigraphy that is long and complex. Fourth, there are numerous supposed 'tools' made of bone, limestone, bauxite or quartz. The vast majority of these 'tools' cannot be proved to be artefacts — but some of the quartz tools are undoubtedly humanly worked. Lastly, the cave has clearly been closed for a long time but it is unlikely that any cave would remain closed for 500,000 yr as has been claimed.

This brings us face to face with the problem of dating. Most now agree that the initial estimate of 70,000 yr BP is too young and that on a variety of grounds a date in excess of 200,000 yr BP is more likely. The dating claimed by Poulianos of over 700,000 yr BP for the skull is very hard to accept on the evidence produced at this meeting. Perhaps the most impressive speakers on dating were M. Ikeya, the originator of ESR dating, and Dr Yokoyama. The consensus of their views would seem to be that 400,000 yr BP would be an appropriate date for Layer 11, the layer said by Poulianos to correlate with the deposits associated with the skull; but this stratigraphical correlation is difficult to prove over the distance involved in the cave. Various other claims relate to the occurrence of fire at one million years BP, the discovery of the true entrance to the cave used by Petralona man and the recovery of fragments of the Petralona man's postcranial skeleton from the 'Mausoleum' area. Of the latter claim, I can speak personally, having been allowed to examine, with several others, the supposed postcranial fragments. We agreed that there was no single identifiable fragment of this sample that was human. The remaining fragments were too small for either identification or attribution.

In anthropological terms, the quarrels that this site has provoked have all the makings of a Greek tragedy as harrowing and as long as the *Medea* of Euripedes performed so well for the delegates at the open-air theatre at the site. Indeed, the site and the situation now require a cool and informed assessment, perhaps by a commission under the chairmanship of Jan Jelinek, the newly elected President of the European Anthropological Association. Such a commission could consist of an internationally respected small group including a palaeontologist, a palaeolithic archaeologist, a palaeoanthropologist, a cave stratigrapher and a geochronologist. Their task could be to examine the bones, the tools, the cave and the claims made for them.

Make no mistake, however, Aris Poulianos and his son, Nickos Poulianos, are to be congratulated on the progress that they have made at the site, but they must also accept that today such a tremendous enterprise must be a team effort. No single researcher can encompass all the disciplines that the rigorous investigation of a site demands today, neither can one man have the time and resources to 'go it alone' successfully.

The view that emerged from the conference was quite clear in that the Petralona specimen is an extremely important skull, almost certainly of Middle Pleistocene age, and of critical importance in documenting the transition from the *Homo erectus* stage of human evolution to the *Homo sapiens* stage in Europe. We owe it to Petralona man, therefore, to gain from the skull and the site every scrap of evidence available to us by the use of modern methods and scientific cooperation. □

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100 years ago

ELABORATE preparations were made in various parts of America to observe the transit of Venus yesterday. The Western Union Telegraph, to facilitate observations, arranged to transmit Washington time wherever desired, in order to secure accuracy in recording results. Some enthusiastic astronomers had proposed general prayer in the churches on Sunday last for clear weather.

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Mysteries of the ringed planets

from J. N. Cuzzi

THE colloquium* on planetary rings recently held at Toulouse was designed as a cross-disciplinary meeting of planetary scientists and astrophysicists that would discuss what is known about ring systems and evaluate theoretical understanding of flattened rotating systems in general. The observational material was, not surprisingly, spacecraft-dominated, but the contributions of ground-based observations have been, and will continue to be, of fundamental importance. Theoretical work comes predominantly from gravitational mechanics, and is now moving towards an understanding of collective effects via both fluid-mechanical and statistical-mechanical pathways. Studies of electromagnetic phenomena are also of great importance.

Voyager observations of Saturn's rings and accumulating data from stellar accultations by Uranus's rings provided most of the new observational material. Jupiter's faint ring, first suspected to exist after Pioneer 10 and 11 encounters in 1973 and 1974, was last closely examined by Voyager in 1979. The three known ring systems exhibit certain family resemblances, such as in their proximity to their parent planet and magnetospheric environment; Saturn and Uranus both possess narrow, eccentric rings with abrupt boundaries, and both Saturn and Jupiter possess broad diffuse low-capacity rings composed substantially of short-lived micrometre-sized particles. There are, however, important differences in the composition and sizes of the constituent particles. The rings of Jupiter and Uranus are composed of quite dark, distinctly non-icy material (Nicholson, Cornell University), whereas the bulk of Saturn's much more extensive rings is composed of water-ice. One reported observation of a ring around Neptune remains unconfirmed, there being uncertainty in the position of Neptune's pole (Elliot, MIT). New results on an even larger 'ring' — the asteroid belt — show it to consist of several distinct sub-rings with differing amounts of dry and water-bearing material (Tedesco, JPL).

One fascinating question is that of the relationship of planetary rings to associated 'moonlets', or objects of 1–100 km size. The observed close association and structural influence of such objects, observed for Saturn and inferred for Uranus, have important implications for the origins of the various rings and, perhaps, for the origin of the Solar System as a whole. The Saturn system is generously endowed with moonlets outside the rings. Charged particle data from Pioneer 11 (Van Allen, University of Iowa) and Voyager imaging data (Terrile, JPL) suggest that two of the larger moonlets, the satellites 1980S26 and 1980S27, which apparently confine or 'shepherd' the curiously kinked and stranded F ring, may be only the largest of many smaller moonlets lying in a broad band about 1,000 km wide. It is probably close encounters with some of these moonlets which kink and braid the F ring, but the details of the process are not fully understood either observationally or theoretically (Showalter, Cornell University).

So far no moonlets have been discovered within the main rings, despite careful searches of promising, but limited, regions by Voyager imaging. The presence of large numbers of 1–10 km-sized objects in the rings would clear gaps of comparable width, which seem to have been ruled out by stellar accultation measurements (Esposito, LASP). Furthermore, radio accultation measurements of ring-scattering properties (Marouf, Stanford University) show directly a fairly sharp upper-size cutoff at 5 m, with a broad, possibly power law-size, distribution extending down to about 1 cm. However, the rings may still contain mountain-sized chunks of matter in numbers far less than the upward extension of the power law would indicate. Several have recently been inferred to lie within the 320 km-wide Encke division. This practically empty region is known to contain a few discontinuous clumpy ringlets similar in appearance to the 'kinky' F ring, and gravitational effects by other hypothetical moonlets have been invoked by analogy. New analysis of Voyager images has also revealed azimuthal waves running along the inner and outer edges of the division in orderly patterns which may reflect recent passage by (still unseen) small moonlets (Cuzzin and Scargle, Ames). This is, of course, also indirect evidence but it has a firmer theoretical base. Confirmation of the existence of such moonlets in the rings would help decide between alternative theories of the origin of the rings.

It is becoming increasingly apparent that the large-scale structure of the rings (A, B, C and so on) is highly meaningful, independent of the detailed behaviour of fine internal structure discussed below. In studies of typical length scales from stellar occultation data, Esposito has found no scales preferred over noise with the possible exception of the very largest scales, and has suggested that the significant dynamical processes act on scales far larger than any discussed so far. Combinations of radio opacity (Tyler, Stanford University) and visible-wavelength opacity show that the particle size distribution is significantly different in the A and B rings from that in the optically thinner C ring and Cassini division; this is in addition to the already known regional differences in particle colour and brightness. Although little attention was given at the meeting to the various origin scenarios, R. Hart (Johns Hopkins University) drew attention to the formative role of the initial energy and momentum of the material, which might also have determined the overall distribution of matter in the planetary system. However, significant dissipative processes may have obscured initial conditions, and ongoing formative processes must be better understood before more detailed inferences as to origin may be drawn.

The question of the fine structure in the rings is yielding to study, but only slightly. The strongest gravitational resonances in the rings are readily identifiable with structure. The two strongest are responsible for the outer edges of the A and B rings, which are radially very sharp ($\leqslant$ 100m), and may be comparable in scale with the vertical height or ring thickness. The sharpness of these edges can be understood in terms of local resonant distortion of the particle rotation law from Keplerian, which may allow diffusive transport of angular momentum to vanish (Goldreich, CalTech).

Further detailed calculations (Borderies, CalTech) verify the amplitude of the distortion of the edge of the B ring as due to distortion of resonant streamlines by self-gravity. Weaker resonances produce Lin-Shu spiral waves of either density or vertical displacement at dozens of locations in regions of moderate optical depth, much as predicted by Goldreich and Tremaine several years before Voyager. Almost all the fine regular structure in the outer A ring is probably due to similar, but unresolved, features in excellent positional agreement with predicted resonances with 1980S26 and 1980S27, with the notable exception of the narrow gap near 2.26 Rs, now named Keeler by the IAU. Spiral waves enable the local mass density to be determined and their damping may be used with caution to measure the local viscosity (Lissauer, Berkeley), yielding an estimate of the local vertical scale height of tens of metres in the outer A ring. The question of ring scale height continues to generate controversy; however, a scale height of tens of metres, in a ring composed of centimetre-to-metre-sized particles, would satisfy the 'many-particle-thick' concept favoured by remote-sensing observations such as the opposition brightening (Irvine, University of Massachusetts), radar backscatter (Pettengill, MIT) and radio occultation (Marouf, Stanford University).

In the optically thinner C ring, strong resonances produce very different structure, for reasons not understood. Optically thick ringlets seem to form,

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*'Planetary Rings', IAU Colloquium number 75, was organized by A. Brahic (Meudon) and R. Greenberg (PSI) and held on 30 August–2 September 1982

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usually within or adjacent to fairly clear gaps. Both the ringlets and the gaps show intriguing and unexplained fine structure down to kilometre scale (Lane, JPL). Resonances with Jupiter in the optically even thinner asteroid belt appear to have acted primarily to clear the Kirkwood gaps, but only following the clearing of any massive primordial nebula (Murray, Cornell University). More detailed attempts to assign resonances to the many other features in the C and B rings have been less successful. The enormous number of features calls for comparison with much more numerous, but much weaker, resonances (Wiesel, USAF Institute of Technology). It has been argued alternatively that the uncounted, randomly distributed narrow features in, at least, the B ring may result from a local 'diffusion' or viscous instability, and not from resonances at all. However, early versions of this theory predicted more dramatic fluctuations than observed. Recent refinements have used theoretical understanding gained from dynamical studies of the evolution of particle disks, pioneered by Safronov and recently extended numerically by Brahic, Casanave (CNES) and Clairemidi (Besançon), and analytically by Hornung and Pellat (Ecole Polytechnique). Such results generally indicate different random velocities for particles of different sizes; a sort of 'thermal equilibrium' with the less massive particles being hotter. This refinement appears to smooth the instability-predicted optical depths into better agreement with the observations (Ward and Harris, JPL). However, the problem of the myriad ringlets appears to be a long way from solution.

The narrow eccentric ringlets of Uranus are being explored in increasing detail by occultation measurements in the near-IR where suitable stars are more plentiful and scattered light is limited by methane absorbtion in the planet's atmosphere. The information has made it possible to determine not only ring eccentricities but also inclinations of comparable amplitude. The eccentricities and inclinations both show a large systematic decrease with increasing distance from the planet, with the exception of the unusually broad and eccentric or outer ring (French, MIT). The observations are, in fact, accurate enough for satellite perturbations of Uranus itself from the system barycentre to be detectable, and direct detection of the influence on the rings of shepherding satellites (Tremaine, MIT), such as, perhaps, distortion of the type seen along the edges of the Encke division, should soon be possible. Certainly the many stellar occultations which will be obtained during Voyager 2 encounter will be extremely illuminating. It is becoming increasingly difficult to make confident assignments of the uranian rings themselves to any three-body resonances once thought appropriate (Aksnes, University of Trömso; Tremaine). Postulated shepherding moonlets could, however, be locked in nearby resonant orbits. Because most known narrow ringlets have nearly the maximum eccentricity allowed before orbits start to cross, and because collisions in the rings are common and dissipate angular momentum, postulated shepherding satellites must be nearby to maintain the eccentricity (Tremaine).

In an extensive discussion of gravitational torques and angular momentum transfer, Greenberg stressed the importance of damping processes, which are implicitly assumed in current shepherding formalisms, but which may require special attention in limiting cases of low optical depth or strong interactions. Goldreich also discussed the role of dissipation from the perspective of streamline distortion, and of the tendency of strong perturbations to modify the rotation law sufficiently to cause the normal outward flow of angular momentum to cease or even reverse. Further progress towards understanding the true 'efficiency' of strong resonances and gravitational torques in general may help us to understand at least one puzzle — why the close-in 'ringmoons' are not being hurled away from the rings by their own resonances.

The importance of electromagnetic effects is well established for Saturn's rings, and probably for Jupiter's ring as well. The activity of the 'spokes' in Saturn's rings has been shown to display the periodicity of Saturn's magnetic field, and maximum activity is more specifically associated with the anomalous magnetic longitude on the planet which is, for reasons unknown, also responsible for Saturn's own kilometre-wavelength radiation (Porco, CalTech). Although most spoke features rotate at the local orbital rate, certain sharp nearly radial edges seem to be more nearly co-rotating with the magnetic field (Terrille), perhaps implying ongoing formation. Theoretical work is sketchy but promising, generally focusing on instabilities in current loops running between the rings and the ionosphere (Goldreich), perhaps with subsequent development of propagating clouds of plasma which serve to levitate pre-existing dust grains away from particle surfaces (Morfill, Max-Planck Institut, Garching). Whether the mysterious static, or SED, discovered by the Voyager Planetary Radio Astronomy experiment is connected with the rings (Warwick, Radiophysics, Boulder) or planetary lighting (Burns, Cornell University) remains a subject of lively debate; further observational constraints on power spectrum and periodicity are being obtained (Zarka, Meudon), ground-based detection is being sought (LeCacheaux, Paris Observatory) and radio occultation results will eventually be capable of addressing the question of a gap at the proper location identified by one, but not both, Voyager stellar occultation experiments. Theoretical studies of electromagnetic effects on the behaviour of micrometre and smaller ring particles (Grün, M-P, Heidelberg; Mendis, UCSD) indicate that they have a significant role in the Jupiter ring, perhaps determining the morphology of its 30,000 km-thick 'halo', and probably also in the outer tenuous E, F and G rings of Saturn. The E ring is unlike its neighbours in colour and apparently consists largely of short-lived micrometre-sized particles. This broad ring is centred very close to the orbit of the 250 km-radium moon Enceladus, which itself shows unique surface evidence of recent geological activity, suggesting Enceladus provides the source of ring particles in some way (Johnson, Pang; JPL). The E and G rings are more vertically extended than the main rings, about 1,000 and 100 km respectively (Wassermann, Lowell; Grün, Pedersen), which may be due to electromagnetic effects. However, the problem of the behaviour of charged grains in all of these ring processes is complicated by the stochastic and fluctuating nature of grain charges, both in magnitude (Lafon, Meudon) and in sign (Meyer-Vernet, CNRS).

Many of the processes illustrated in existing ring systems may have been active during the formation of the planets. Safronov (Schmidt Institute) reviewed recent work on nebular evolution. The essence of the problem is transfer of angular momentum as the interstellar medium collapses to form a solar system. The role of viscosity is central; slightly different assumptions as to its source and nature producing qualitatively different results in evolution calculations. Controversy remains as to whether planets formed through such gradual accumulation of planetesimals in a viscous collisional disk or by gravitational instability of nebular gas and dust with subsequent loss of light components. Even electromagnetic effects may have had their day very early in the game. Safronov believes that the very existence of planetary rings, each 'frozen' within its parent planet's Roche zone, attests to the once-ubiquitous presence of a particulate protoplanetary disk.

In summary, although observational and theoretical progress is impressive, there are still many outstanding and intriguing problems. Connections between various ring systems are becoming more apparent. Theoretical studies initiated to understand observed phenomena are aiding basic research into the behaviour of protoplanetary disks. It became clear at the meeting that considerable progress both in theory and observation, could take place in the near future. Further in-depth analysis of Voyager data and a strong continuing programme of ground and orbit-based observations are necessary, and it is hoped that the time will soon again be ripe for further close observation by spacecraft. □

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REVIEW ARTICLE

Molecular mechanisms of general anaesthesia

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Important constraints on possible molecular mechanisms of general anaesthesia are derived from a quantitative reappraisal of data on the potency of general anaesthetics on whole animals. Despite their popularity, theories that invoke lipids as the prime target do not look at all promising, and available data point much more plausibly to a direct effect on particularly sensitive proteins. Structural changes of proteins on binding general anaesthetics are probably small but may be sufficient to perturb normal function; alternatively, anaesthetics may compete with an endogenous ligand. The phenomenon of pressure reversal of anaesthesia may simply be due to anaesthetics being squeezed away from their target sites.

MODERN surgery would be inconceivable without general anaesthetics. Yet, after more than a century of research, there are very few points of agreement about how and where they act. The extraordinary diversity of theories which have been proposed over the years is matched only by the diversity of molecules which can induce reversible loss of consciousness. Indeed, general anaesthetics form no single chemical class but include substances as dissimilar as xenon, chloroform and thiopentone. This apparent lack of specificity, together with the remarkable observation that general anaesthesia can be reversed by high pressure, poses a unique pharmacological problem.

Unfortunately, rather than converging to a solution, the field at present seems to be diverging along a number of mutually incompatible lines, with various groups becoming entrenched in the defence of particular theories. It therefore seems timely to review the field critically and examine which ideas, in the light of recent data, are really tenable. We hope to show that, despite the present lack of consensus, there are real grounds for optimism.

In this review, we will first discuss what inferences can be made about the molecular nature of the general anaesthetic site(s) from a quantitative consideration of data on the potency of general anaesthetics on whole animals. With this information in mind, we will then assess the relevance of the numerous studies on simple systems and discuss whether lipid or protein provides the most plausible molecular target for general anaesthetic action. (We will not review the literature on isolated nerve preparations, partly because few unifying themes have emerged but mainly because expert and extensive reviews[1-3] have recently appeared.)

Whole animals

The induction of general anaesthesia must involve a site or a number of sites in the brain. (This is in contrast to local anaesthesia, which results from the blockage of nerve pathways outside the brain.) Because it is not known where in the brain general anaesthetics exert their primary effect, potencies can only be determined using the whole animal. Various behavioural end points, reflecting complex central nervous system activity, have been used and give potencies which usually agree to within a factor of two. Potency is defined as the reciprocal of that concentration (ED_{50}) at which 50% of the animals are anaesthetized, and most quantitative work has rightly focused on the importance of obtaining and analysing accurate ED_{50} values.

Concentrating on anaesthetic end point data effectively means ignoring the large variety of side effects which different general anaesthetics produce and which are of crucial importance to the clinician. The assumption that side effects do not interfere with the anaesthetic end point is, of course, only an approximation; however, a quantitative interpretation of potency data is possible only if this simplifying assumption is made.

What property of the anaesthetic molecule accounts for its potency?

As early as the 1890s, it was known that the potency of an anaesthetic increased roughly in proportion to its partition coefficient (concentration ratio) between olive oil and water[4-6]. We have shown that an even better correlation is obtained using the slightly more polar solvent octanol, whereas very poor correlations are found with apolar alkane solvents[7]. Although there have been many attempts to correlate potency with various other physical properties of the anaesthetic molecule, none of them has been as complete or successful as the correlations with partition coefficients[1,8].

The attraction of these solubility correlations is that they provide a simple physical picture. Since a log–log plot of potency in the aqueous phase versus octanol/water partition coefficient has a unit slope[7], then, if the general anaesthetic site has solubility properties similar to octanol, all anaesthetic molecules would be equally potent at the site. One must be very careful, when comparing potencies, not to get confused between observed values and the potency at the site itself. For example, in air, ether is roughly five times more potent than cyclopropane[9], whereas in water it is about ten times less potent[7]; at an octanol-like site, however, both anaesthetics would be equally potent. It is not necessary to postulate that some anaesthetics are intrinsically more effective than others at the site, due, for example, to their relative abilities to break hydrogen bonds[10] or to their relative molecular volumes[11]. It is also worth pointing out here, while discussing potencies, that a common mistake is to compare[12-14] potencies in one phase (such as air) with partition coefficients between a solvent and another phase (such as water). The remarkable fact that anaesthetic potency can be predicted from such a simple parameter as the octanol/water partition coefficient not only shows that potency is essentially independent of the detailed size, shape and chemical nature of the anaesthetic molecule, but also strongly suggests that the site is amphiphilic, having both polar and apolar characteristics[7,15].

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Some drugs could not be considered in the above correlations because their potencies have not been determined. For example, it is very difficult to determine equilibrium potencies for intravenously administered anaesthetics, because of their complicated pharmacokinetics and the fact that (in contrast to inhalational anaesthetics) an effectively infinite reservoir of drug is not present. Other molecules are excluded for potentially more interesting reasons. As one ascends an homologous series, a point is reached beyond which higher members of the series are not anaesthetic—the so-called 'cutoff' effect[11]. The most obvious explanation for this finding is that the site has a finite size and simply excludes molecules which are too large. While this may reasonably account for decane and tetradecanol being inactive, it is not so immediately obvious why a saturated vapour of C_3F_8 does not anaesthetize mice[16], even after allowing for the much larger volumes of fluorinated compounds[17] compared with their hydrogenated counterparts. What all the 'cutoff' molecules do have in common are very low aqueous solubilities. Indeed, it has recently been suggested[18] that because of the low aqueous solubility, it is impossible, in principle, to deliver enough drug to the site, even at equilibrium. However, the final equilibrium concentration at the site must be independent of path; nonetheless, the time taken to reach equilibrium may be so long that, in practice, the drugs appear inactive. We feel that more work is needed before one can say for certain whether or not the observed 'cutoff' phenomena reflect fundamental properties of the general anaesthetic site.

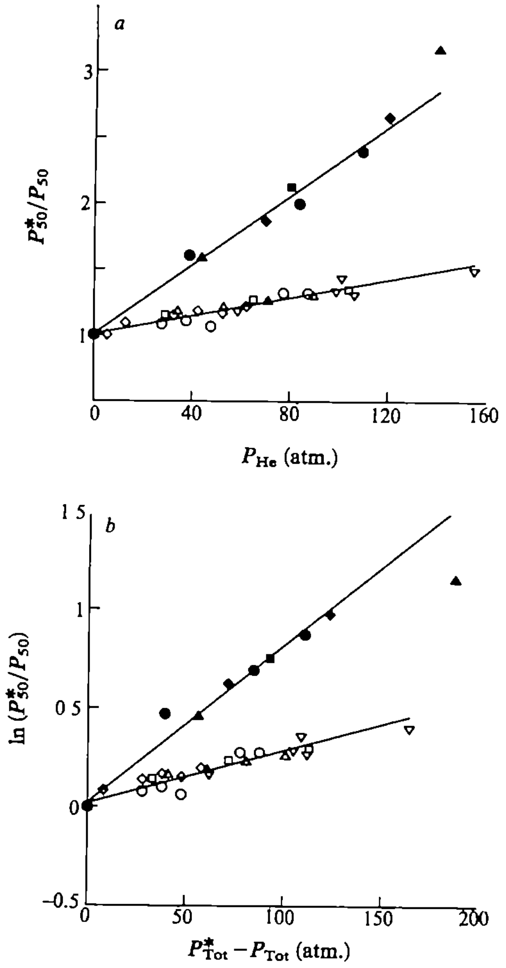

Fig. 1 Pressure reversal data for mice and newts interpreted according to *a*, the critical volume hypothesis[21] and *b*, a simple binding model. P^*_{50} and P_{50} are the anaesthetic partial pressures needed to anaesthetize 50% of a population of animals at total pressures P^*_{Tot} and P_{Tot}, respectively, and P_{He} is the partial pressure of helium used to increase the total pressure. Open symbols are for the mouse, and filled symbols are for the newt. The anaesthetic gases are: N_2O (○, ●); SF_6 (◇, ◆); Ar (▽), CF_4 (□, ■); and N_2 (△, ▲). The data were taken from refs 21 and 22. In *a*, the data are plotted according to the prediction[21] of the critical volume hypothesis $P^*_{50}/P_{50} = \text{constant} \cdot P_{He} + 1$, where the constant factor (the slope of the lines) depends upon the compressibility and molar volume of the site, the fractional volume expansion needed to induce anaesthesia, and the partial molar volume and mole fraction solubility of helium at the site. In *b*, the data are plotted according to the prediction of a simple binding model (see text), derived as follows: Let the equilibrium between concentrations of unoccupied site [S], free anaesthetic [A], and inactive complex [SA] be described by the equilibrium constant $K_{eq} = [SA]/([S][A])$. The thermodynamic relationship between K_{eq} and total pressure P_{Tot} is given[23] by $\partial(\ln K_{eq})/\partial P_{Tot} = -\Delta V/(RT)$, where R is the gas constant, T is the absolute temperature and ΔV is the increase in volume of the system when anaesthetic binds to the site. If we assume that anaesthesia occurs when the ratio [SA]/[S] of occupied to unoccupied sites reaches some critical value, then, by integrating the thermodynamic relationship and assuming ideal gas behaviour, we obtain the prediction that $\ln(P^*_{50}/P_{50}) = (\Delta V/RT) \cdot (P^*_{Tot} - P_{Tot})$.

How does potency vary with pressure?

Few pharmacologists would expect high pressure to be a particularly useful tool when trying to elucidate the mechanism of action of a drug. However, the intriguing observation[19,20] that general anaesthesia can be reversed by high pressures (of the order of 150 atmospheres) provides what might be an important clue as to how anaesthetics act. If pressure exerts its effect at the anaesthetic site, then, since pressure can only act by reducing volume, this implies that the total volume of site plus anaesthetic increases when they interact. Although such an increase in volume may be secondary to the anaesthetic mechanism, there are those[21] who postulate that it is the primary cause. The most self-consistent and best known of these volume expansion theories is the critical volume hypothesis: "Anesthesia occurs when the volume of a hydrophobic region is caused to expand beyond a certain critical amount by the absorption of molecules of an inert substance. If the volume of this hydrophobic region can be restored by changes of temperature or pressure then the anaesthesia will be removed"[8]. According to the theory[21], as the total pressure is raised, more anaesthetic is needed, simply to counteract the reduction in volume caused by pressure. Figure 1*a* shows that the quantitative predictions of the critical volume hypothesis are well satisfied using a range of gaseous anaesthetics, for both newts (filled symbols) and mice (open symbols). The slope can only be interpreted if certain unknown properties of the anaesthetic site are assumed; the different slopes for the two animals are said to be due to different site compressibilities and critical volumes[21,22].

The impressive agreement between the predictions of the critical volume hypothesis and the pressure reversal data for gaseous anaesthetics has been the main reason for its popular acceptance. However, such agreement is only a necessary but not a sufficient condition for the validity of the hypothesis, and other, more molecularly plausible, theories might fit the data equally well. Perhaps the simplest molecular model is one in which a single anaesthetic molecule binds to the site and inactivates it. This model can be quantitatively tested with the same data used to support the critical volume hypothesis. When this is done (see Fig. 1*b*), we find, remarkably, that this simple binding model accounts for the pressure reversal data as convincingly as the critical volume hypothesis (see Fig. 1*a*).

An advantage of this simple binding model over the critical volume hypothesis is that the slopes of the lines in Fig. 1*b* can be interpreted directly in terms of a single molecular parameter (ΔV), the average partial molar volume of the anaesthetic gases at the site. (Had the potencies been expressed in terms of aqueous concentrations, then ΔV would be the *difference* between the volumes at the site and in water.) We calculate $\Delta V \simeq 190\ \text{cm}^3\ \text{mol}^{-1}$ at the newt site and $\Delta V \simeq 65\ \text{cm}^3\ \text{mol}^{-1}$ at the mouse site. These values can be compared to an average partial molar volume of about $65\ \text{cm}^3\ \text{mol}^{-1}$ for these anaesthetics in a simple solvent such as benzene[21,22]. The larger value for the newt may indicate that, in addition to the expansion caused by simple solvation of the anaesthetic molecule, there is some additional volume increase due to a small change in the structure of the site itself. (If the site were a protein of weight 100,000 g mol^{-1} which undergoes a conformational change on binding[24], an expansion of only about 0.2% would be sufficient to account for the newt data.) Alternatively, the larger volume could be due to more than one anaesthetic molecule combining with the newt's site. For the mouse, on the other hand, the value of ΔV for these gases is about the same as their average partial molar volume in simple solvents and can thus be accounted for by simple solvation, with little or no additional volume change due to disruption of the site.

It is very often stated that pressure reversal cannot be due to simply squeezing the anaesthetic molecule away from its target site. Our above analysis shows that this simple notion *should not be dismissed.* Indeed, when one considers the mouse data in Fig. 1*b*, it is difficult to imagine any site—be it protein or lipid—in the mouse (or presumably in man) for which this would not be the major factor!

The appealing simplicity of the pressure reversal data with gaseous anaesthetics has not been matched by more recent results obtained using nongaseous anaesthetics injected into mice and rats. For these compounds, the decrease in potency with increasing pressure is usually greater[25] than that observed with the gases in Fig. 1, can vary significantly from drug to drug[26] and can even be nonlinear[26]. However, we feel that the experimental complications associated with the use of intravenous agents should not be underestimated, and perhaps these results would have been on a surer footing had they been obtained with animals (such as tadpoles) having simpler pharmacokinetics[25,27].

Finally, although it is possible to explain most of the pressure-reversal data by assuming that pressure and anaesthetics act at the same site, the possibility that they act at different sites must be seriously considered. For example, pressure reversal could be due to pressure causing a general increase in excitability, which counteracts a general decrease in excitability caused by anaesthetics[28,29].

How does potency vary with temperature?

Temperature is a much more familiar experimental variable than pressure, and the effects of temperature on anaesthetic potency have been measured for a number of animals. Nonetheless, the importance of these results has often been underestimated, due, we feel, to the fact that they do not agree with the predictions of most current theories of general anaesthesia.

In 1901, Hans Meyer[5] found that the aqueous potencies for tadpoles sometimes increased and sometimes decreased with temperature, depending upon the anaesthetic, but that these changes could be largely accounted for by corresponding changes in the oil/water partition coefficients. Later, Kurt Meyer and Hemmi[30] reached the same conclusion. More recent work with goldfish[12] showed that potencies expressed in terms of partial pressures decreased markedly with increasing temperature, in line with similar, but somewhat smaller, changes in oil/gas partition coefficients[31,32]. (It should be stressed that, although oil/water partition coefficients can either increase or decrease with rising temperature, oil/gas coefficients usually decrease[31].)

In agreement with the goldfish results[12], Eger and his colleagues[32–34] found that gas phase potencies for dogs also consistently decreased with increasing temperatures. An obvious problem when working with dogs, or indeed with any warm-blooded animal, is that deviations from the normal body temperature may well produce physiological effects which interfere with the anaesthetic endpoint. At extreme temperatures, this was clearly the case. Over a moderate temperature range, however, this did not appear to be a major complication. Not only were relative changes in potency quite different for different anaesthetics, but for the least soluble anaesthetic (cyclopropane) the change was minimal and could be almost entirely accounted for by changes in solubility. Furthermore, potency changed in the same manner both above and below the normal body temperature (see Fig. 2).

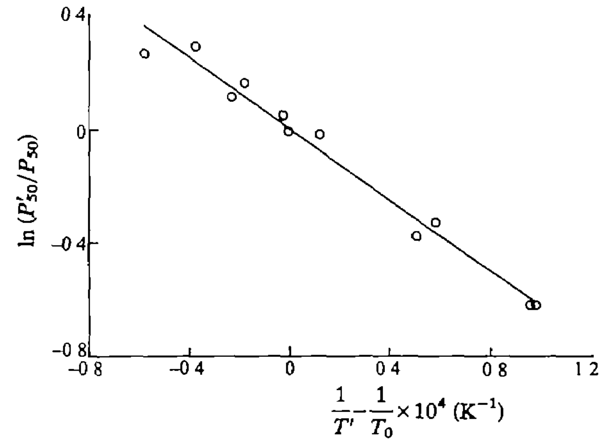

Fig. 2 The effect of temperature on the potency of halothane for dogs, interpreted according to a simple binding model. P'_{50} is the partial pressure of halothane needed to induce anaesthesia at a body temperature T', and P_{50} is the partial pressure at the normal body temperature $T_0 = 310$ K (37 °C). The data[32–34] are for dogs with body temperatures T' between about 28 and 43 °C. According to a simple binding model, in which a single anaesthetic molecule binds to the site and inactivates it, the equilibrium between concentrations of unoccupied site [S], free anaesthetic [A], and inactive complex [SA] can be described by the equilibrium constant $K_{eq} = [SA]/([S][A])$, which will vary with temperature according to[23] $\partial(\ln K_{eq})/\partial T = \Delta H/(RT^2)$, where ΔH is the enthalpy of binding, that is, $-\Delta H$ represents the heat released when anaesthetic binds to the site. If we assume that anaesthesia occurs when some constant fraction of sites are inactivated, then integration between T' and T_0 yields the prediction that $\ln(P'_{50}/P_{50}) = (\Delta H/R)\cdot(1/T' - 1/T_0)$. For these data, the slope of the line gives $\Delta H = -52$ kJ mol^{-1}.

The temperature results pose a serious problem for some popular theories (for example the critical volume hypothesis and lipid bilayer fluidity and phase transition theories), which predict that a rise in temperature should contribute positively to the anaesthetic end point. They can, however, be readily explained in terms of the simple binding model introduced in the previous section. This is shown quantitatively in Fig. 2, where we have plotted the most extensive set of published data (for halothane and dogs). From the slope of the line, we calculate that 52 kJ mol^{-1} of heat is released when halothane binds to the dog site from the gas phase. Heats of binding for various inhalational anaesthetics lie in the range 16 to 56 kJ mol^{-1} for the dog[32,33] and goldfish[12], with good agreement for a given anaesthetic. The heat released is comparable with, but consistently larger than, that released when binding to olive oil[31–33] and, although the values for oil are not particularly accurate, the difference is probably real. It might represent some interference with the anaesthetic end point by temperature itself or, more interestingly, it might reflect a genuine difference in binding, indicating that an anaesthetic is more tightly bound at the site than in a simple solvent. Whatever the precise details of the thermodynamics, it is clear that binding models quite naturally account for the consistent experimental observation that gas phase potency decreases with increasing temperature, because of the tendency of heat to drive the anaesthetic off the site into the gas phase.

Molecular models of the anaesthetic site

In a field in which there is so much controversy, it is perhaps comforting to find at least one point of almost universal agreement. Most workers feel that the *ultimate* effects of general anaesthetics are on proteins. The question is: do anaesthetics act directly on proteins, or do they act indirectly by first affecting membrane lipids? An attraction of the idea that membrane lipids may be the primary site is that anaesthetic potencies would be easy to account for[35], since both aqueous potencies[7] and lipid/water partition coefficients[36] correlate well with octanol/water partition coefficients.

Lipid bilayer fluidity. Soon after the introduction of magnetic resonance techniques to the study of membranes, it was shown[37] that anaesthetics could increase the fluidity of membranes and lipid bilayers. These results led to the popular theory that anaesthetics act by increasing the fluidity of membrane lipids, which, in turn, perturbs the normal functioning of some crucial membrane protein, such as a nerve channel. A large number of subsequent studies, using a variety of techniques, apparently lent support to this idea. In retrospect, however, it is clear that, almost invariably, very high concentrations of anaesthetics were used to obtain the observed increases in fluidity[7,38]. More recent work[7,38–41] has shown that, at clinically relevant concentrations, changes in lipid bilayer structure and fluidity are virtually undetectable. The few reports that fluidity is significantly altered at surgical concentrations are either contradictory[42,43] or else have been obtained at higher than the minimum alveolar concentrations[44].

Thus purely quantitative considerations argue against changes in bilayer fluidity being important. Nonetheless, it has been argued[45] that measurements at high concentrations, where clear effects are seen, are relevant and can be extrapolated back to clinical concentrations. This is a risky procedure, since it is extremely difficult to demonstrate linearity of response at low anaesthetic concentrations, where barely measurable effects occur. Interesting information can, however, sometimes be obtained. Such experiments have shown that high concentrations of anaesthetics can sometimes order and sometimes disorder, depending upon the anaesthetic and spin-probe used as well as on the composition of the lipid bilayer[18,45–47]. While this diversity of observed effects has been used[46] to argue against fluidizing theories, it has also, on the other hand, encouraged proponents of such theories to 'fine-tune' their systems so that consistent responses are observed with all anaesthetics. Following the latter philosophy, Miller and Pang[45] concluded that a suitable bilayer must contain at least 10% (molar) cholesterol and have a charge density of between 0.12 and 0.46 charges per phospholipid.

At surgical concentrations, however, fluidity increases in such bilayers (estimated by extrapolation) would be exceedingly small. Indeed, equivalent increases in fluidity occur in cholesterol-containing bilayers which have been warmed only a few tenths of a degree[41,48]. Needless to say, such small changes in temperature do not induce anaesthesia in whole animals.

$$\begin{array}{ccc} (a) & & (b) \\ \mathrm{E} \underset{}{\overset{K_1}{\rightleftharpoons}} \mathrm{E_I} & \begin{array}{c}\text{Thermal}\\ \text{inactivation}\end{array} & \mathrm{E} \overset{K_1}{\rightleftharpoons} \mathrm{E_I} \\ \mathrm{E_I} + n\mathrm{A} \overset{K_2}{\rightleftharpoons} \mathrm{E_I}n\mathrm{A} & \begin{array}{c}\text{Anaesthetic}\\ \text{binding}\end{array} & \mathrm{E} + n\mathrm{A} \overset{K_3}{\rightleftharpoons} \mathrm{E}n\mathrm{A} \end{array}$$

$$\text{where } K_1 = \frac{[\mathrm{E_I}]}{[\mathrm{E}]}, \qquad K_2 = \frac{[\mathrm{E_I}n\mathrm{A}]}{[\mathrm{E_I}][\mathrm{A}]^n} \quad \text{and} \quad K_3 = \frac{[\mathrm{E}n\mathrm{A}]}{[\mathrm{E}][\mathrm{A}]^n}$$

Fig. 3 Two alternative schemes to explain the inhibition of luciferase by inhalational anaesthetics. In both schemes, the active form (E) of the enzyme is in reversible thermal equilibrium with an inactive form ($\mathrm{E_I}$). The schemes differ only in regard to which of these forms the n anaesthetic molecules (A) are assumed to bind. In *a*, binding occurs only to the thermally denatured form[107], while in *b*, binding occurs to the active form of the enzyme. Both schemes fit the data[107] equally well and give reasonable values for the enthalpy increase ($\Delta H_1 = 320\ \mathrm{kJ\ mol^{-1}}$) and entropy increase ($\Delta S_1 = 1075\ \mathrm{J\ K^{-1}\ mol^{-1}}$) upon thermal denaturation, and they show that about one anaesthetic molecule ($n \sim 1$) binds to each luciferase molecule. Scheme *a* yields extremely large changes in enthalpy ($\Delta H_2 = -335$ to $-376\ \mathrm{kJ\ mol^{-1}}$) and entropy ($\Delta S_2 = -1{,}160$ to $-1{,}340\ \mathrm{J\ K^{-1}\ mol^{-1}}$) upon binding of the various anaesthetics from the gas phase. It is easy to show that $\Delta H_3 = \Delta H_1 + \Delta H_2$ and $\Delta S_3 = \Delta S_1 + \Delta S_2$, so that scheme *b* yields much smaller and more reasonable values ($\Delta H_3 = -15$ to $-56\ \mathrm{kJ\ mol^{-1}}$ and $\Delta S_3 = -85$ to $-265\ \mathrm{J\ K^{-1}\ mol^{-1}}$), consistent with simple binding.

Moreover, fluidization theories predict an increase in anaesthetic potency with rising temperature (since fluidity increases markedly with temperature), whereas, as we have seen above (Fig. 2), the reverse is observed experimentally. At first sight, one of the attractions of these theories is that pressure might reasonably be expected to counteract the fluidizing effect of anaesthetics, so that pressure reversal seems easy to explain. While qualitatively this prediction is supported by experimental observations, quantitatively the agreement is very poor. The fluidity increases at concentrations which cause general anaesthesia would be counteracted by about 25 atmospheres or less[39,49,50], whereas pressures of the order of 150 atmospheres are necessary to overcome anaesthesia in the whole animal. In summary, it seems to us most improbable that changes in lipid bilayer fluidity play any significant role in general anaesthesia.

Phase transitions in lipid bilayers. Another class of lipid theories supposes that certain bilayers in nerve membranes are poised near a chain-melting phase transition, and that anaesthetics induce a cooperative structural change. Most general anaesthetics do in fact significantly depress the temperature T_m of the gel to liquid crystalline phase transition in pure lipid systems at clinical concentrations[51–53]. However, the observed effects are equivalent to those produced by a temperature increase of less than a degree[51–53] and can be reversed by only a few tens of atmospheres of pressure[52,53]. Also, some anaesthetics (the higher alcohols) can actually increase[46,54,55] T_m. Thus these theories suffer from most of the shortcomings of the fluidization theories. An attempt has been made to 'fine-tune' phase transition theories, by introducing lipid mixtures which undergo lateral phase separation[56]. However, not only do difficulties with temperature remain, but two equally potent anaesthetics have been shown[18] to shift solid/fluid phase equilibria in opposite directions.

Finally, it should be remembered that there is little convincing evidence for the existence of significant regions of solid-phase lipids in mammalian membranes. Indeed, in view of the high concentrations of unsaturated hydrocarbon chains in these membranes and the high concentrations of cholesterol in nerve plasma membranes in particular, one would need a large measure of faith to remain convinced that the answer to general anaesthesia lies in the study of lipid phase transitions.

Changes in membrane dimensions. When anaesthetics partition into membranes, some geometrical changes are almost certain to occur. Early workers[57–59] found that clinical concentrations of general anaesthetics produce sizeable changes in the surface pressure of lipid monolayers held at a constant area at an air/water interface, and these correspond to smaller but still significant increases in area (of about 0.5%)[60] at constant pressure. More importantly, very similar increases have been observed in the area of intact red blood cell membranes[61].

The effect of anaesthetics on membrane thickness is much more controversial. Using X-ray and neutron diffraction, we could detect no significant change in the structure or thickness of lipid bilayers exposed to surgical concentrations of nitrous oxide, halothane or cyclopropane[7,38]. Haydon and his colleagues, on the other hand, showed that high concentrations of the lower alkanes cause significant decreases in the electrical capacitance of black lipid films[62] and squid axon membranes[63], which they reasonably interpreted as thickness increases. Alkanes have also been observed to increase bilayer thickness in multiwalled lipid vesicles[64]. A large increase in thickness (a capacitance decrease) was also reported to occur in solvent-containing black films exposed to benzyl alcohol[65]. However, this report now appears to have been misleading, since later workers[66–68] have shown that, when 'solvent-free' films are used, benzyl alcohol, octanol and chloroform actually increase the capacitance. While this might suggest that these agents decrease bilayer thickness, the observed increases in capacitance could be largely due to increases in dielectric constant[68]. Looking at all available results, on fluid lipid bilayers[7,38,40,66], 'solvent-free' black films[66–68], and myelin membranes[69], and extrapolating where necessary to low concentrations, we are forced to conclude that any changes in membrane thickness at general anaesthetic concentrations must be very small.

There has been considerable confusion about the size and importance of the volume changes that occur when anaesthetics enter membranes. On the basis of area expansion and mass density measurements[61,70,71], Seeman calculated that while lipid bilayers expand by only the van der Waals volumes of the occupying drug molecules, biological membranes expand by about 20 times this amount. We have recently reinvestigated this problem and find that, in fact, the volume expansion caused by a molecule of halothane is essentially the same in both red cell membranes and lipid bilayers, and little different from that in liquid halothane or water[72]. Kita *et al.*[73] have also measured these volumes for a number of different lipid bilayers and anaesthetics and found values close to those in the pure liquid anaesthetics but slightly greater than those in water. In terms of total membrane volume, both red cell membranes[72] and lipid bilayers[72,73] expand by only a few tenths of a per cent at clinical concentrations.

In summary, small increases in both area and volume have been detected at clinical concentrations of general anaesthetics, but whether or not changes in thickness occur is uncertain. An estimate of the thickness change can be made, since a small fractional change in volume must equal the sum of the fractional changes in area and thickness. For red cell membranes and halothane, the observed volume expansion[72] of about 0.1% and area expansion[61] of about 0.4% imply a thickness decrease of about 0.3% (less than 0.2 Å). The possibility of anisotropic changes in membrane dimensions of this sort has been discussed by others[21,74]. What is important, however, is that changes in membrane dimensions at clinical concentrations are very small indeed. (Comparable changes are produced by only slight increases in temperature: area[75], thickness[76] and volume[77] all change by $\geqslant 0.1\%$ per degree.) Thus we feel that changes in membrane dimensions do not provide a very plausible basis for theories of general anaesthesia.

Permeability changes in lipid bilayers. Probably the most promising line of argument for those who believe that anaesthetics act primarily on lipids would rest on the effects observed on bilayer permeability. Bangham and his co-workers[59,78,79] showed that the leakage of cations from lipid vesicles is markedly enhanced by general anaesthetics and that these changes can be reversed by high pressure. At clinical concentrations, these permeability increases can be as large as 20–50% for cations[80], but, interestingly, appear to be much smaller for anions[68] and non-electrolytes[68,79]. Although the permeability of lipid bilayers to inorganic cations is very small, and orders of magnitude less than that observed in biological membranes, similar effects of anaesthetics and pressure have been observed on the much larger cation fluxes found with ionophore-containing bilayers[78–80].

While these results are certainly tantalizing, it is not obvious that cation fluxes mediated by ionophores such as valinomycin and gramicidin A are appropriate models for ionic movements across natural membranes. Recent experimental results[81], however, suggest that proton permeabilities across pure lipid bilayers may be anomalously high, and Bangham and Mason[82] have proposed that anaesthetics act by affecting these proton movements. They suggest that anaesthetics act by collapsing *p*H gradients across catecholamine-containing synaptic vesicles. This in turn neutralizes the catecholamines (which had been charged in the acidic interior and thus effectively trapped[83]) and allows them to escape, thereby inhibiting synaptic transmission. Convincing experimental evidence in favour of this new and ingenious theory is still rather sparse, and whether or not lipid bilayers are highly permeable to protons is an extremely controversial point[84,85].

Proteins. At first sight, the idea that anaesthetics act directly on protein might seem improbable, since the well constrained structures of most proteins would not be expected to provide the sort of binding environment necessary to account for the simple correlation of anaesthetic potency with octanol solubility[7]. On the other hand, it might be argued that if the anaesthetic site were an integral membrane protein, its necessarily intimate association with membrane lipids might well provide an octanol-like environment at the protein/lipid interface. However, a major experimental problem which arises when working with integral membrane proteins is that it is difficult to disentangle effects on protein from those on the surrounding lipids. While negative results are meaningful, positive results can be difficult to interpret. To avoid these difficulties, we will focus our attention here on the evidence that has been obtained using simple, relatively pure protein systems, where the observed effects can be interpreted reasonably unambiguously in terms of direct protein/anaesthetic interactions.

Quantitative data on the binding of general anaesthetics to proteins are, unfortunately, not very extensive. However, certain anaesthetic alkanes[86,87] and alcohols[87] have been reported to bind to bovine serum albumin (BSA). In addition, Hansch and his colleagues have accumulated a large amount of data on the binding of small organic molecules (including some anaesthetics) to BSA[88] and haemoglobin[89] and have shown that the binding of a molecule can be simply correlated with its octanol/water partition coefficient; although not directly relevant to anaesthesia, these data do illustrate that a diverse range of molecules can bind to a protein in a manner relating to their solubilities in a simple organic solvent. Further, using what data[86–88] are available for the binding of anaesthetic molecules to BSA, we calculate that at concentrations which cause general anaesthesia there would be one or more anaesthetic molecules bound to each protein molecule.

In some cases, organic molecules apparently bind at the interfaces between protein subunits[89] or subunit-like domains[90]. For example, a number of molecules which bind strongly to haemoglobin do not bind measurably to myoglobin[89]. On the other hand, the small anaesthetics xenon, butane and pentane bind strongly to both haemoglobin and myoglobin[91], apparently in the well-defined pockets which have been identified crystallographically by Schoenborn[92]. β-Lactoglobulin[93] and adenylate kinase[94] are other examples of proteins which fortuitously bind anaesthetics in localized pockets. That such pockets will accept molecules only up to a certain size is illustrated by the alkane-binding site of β-lactoglobulin, which can accept two butane molecules equally well, two pentane molecules unequally well, but only one molecule of iodobutane[93]. Although all of these binding studies have been performed on proteins which are obviously not directly involved in general anaesthesia, they do illustrate that the solubility properties of the general anaesthetic site, including the 'cutoff' effect, could be quite plausibly mimicked by a protein.

Binding of an anaesthetic to a protein and inhibition of its function are, of course, two very different things. For example, when anaesthetics bind to haemoglobin, the resulting structural changes are very small[92,95] and the oxygen dissociation curve is unaffected[96]. In contrast to the paucity of data on the binding of anaesthetics to proteins, there is no shortage of reports of direct effects of anaesthetics on protein function. However, if these results are looked at critically, it is clear that in most cases they have little relevance to general anaesthesia: the effects that are or would be observed at clinical concentrations are either very small or undetectable, or else clear effects are seen with some anaesthetics but negligible or opposing effects are seen with others. This is true in most studies with soluble proteins, organized protein assemblies, and membrane proteins[96–102].

One class of proteins, however, which do appear to be particularly sensitive to general anaesthetics are the light-emitting luciferase enzymes. Work with whole cells[103–105] has shown inhibition at low anaesthetic concentrations and even, in some cases, pressure reversal. Although these results are seductive, molecular interpretation is difficult, and there have been too few studies using cell-free systems. A membrane-free preparation of bacterial luciferase has been shown to be inhibited by low concentrations of ether, which apparently competes with the binding of an aldehyde necessary for luminescence[106]. Using a cell-free extract from firefly tails, Ueda and Kamaya[107] showed that firefly luciferase is inhibited by surgical concentrations of halothane, methoxyflurane, chloroform, enflurane and fluroxene. They measured gas-phase potencies at different temperatures but, unfortunately, interpreted their results in terms of a scheme (Fig. 3*a*) inherited from earlier workers[103], in which anaesthetics could only interact with a thermally inactivated form of the enzyme. This scheme gave extremely large decreases in enthalpy and entropy on binding, from which they concluded (we think erroneously) that anaesthetics cause a major conformational change when they bind to luciferase.

However, the luciferase data[107] can be interpreted much more reasonably in terms of a simple binding model (Fig. 3*b*) similar to that introduced in previous sections, in which the anaesthetic binds to the active enzyme and inhibits it. We find that, not only are the enthalpy and entropy changes modest and easy to account for by simple binding (comparable enthalpy changes are observed when these anaesthetics partition from the gas phase into oil[31–33]), but the heats of binding are now similar in magnitude to, and of the same sign as, those found when applying the model to whole animal data. In other words, the temperature dependence of anaesthetic potency in whole animals can easily be accounted for in terms of anaesthetics binding to protein.

Thus, although the data on anaesthetic interactions with proteins are rather sparse compared with those available for lipids, examples of proteins can be found which satisfy most of the properties required of a successful molecular model of the general anaesthetic site.

Conclusions

There are many who would argue that it is premature to search for the molecular mechanisms underlying general anaesthesia. Nonetheless, when one focuses on quantitative data on anaesthetic potencies for whole animals and considers how these potencies vary with pressure and temperature, certain clear trends emerge, which do provide important information on the molecular interactions involved and impose severe constraints on the molecular nature of the target site. For the relatively inert anaesthetics, for which unambiguous potency data exist, these trends are surprisingly simple. In contrast, complications arise with the intravenous agents. While this may indicate that some anaesthetics act differently from others, it may only reflect the more complex pharmacokinetics of these compounds.

Using the constraints imposed by the whole animal potency data, we have assessed the relevance of simple lipid and protein model systems. Despite the vast literature on anaesthetic/lipid interactions, those theories which postulate that anaesthetics exert their primary effects on lipids do not look at all promising, although changes in lipid bilayer permeability remain a possible candidate. In most cases, the results obtained with proteins look equally unpromising; nevertheless, some proteins do exist which are sensitive to surgical levels of general anaesthetics and are inhibited at anaesthetic concentrations which not only correlate well with whole animal potencies, but also show analogous dependencies upon pressure and temperature. Taken as a whole, the data available point much more plausibly to protein rather than lipid as the primary site of action.

We feel that the simplest working hypothesis, best supported by available data, is that general anaesthetics act by binding directly to a particularly sensitive protein (which may or may not be membrane-bound) and inhibiting its normal function. The fact that potencies correlate with octanol, but not hydrocarbon, partition coefficients suggests that the binding site on the protein is amphiphilic. The anaesthetic could inhibit either by interfering directly with catalytic or transport activity (for example by disrupting subunit–subunit contacts) or by competing for the binding of some endogenous ligand (such as a neurotransmitter). In either case, the structural perturbations which occur on binding are probably small, since the volume and enthalpy changes, derived from the effects of pressure and temperature, are modest. Contrary to popular belief, pressure reversal may well be due to pressure simply 'squeezing' the anaesthetic molecules away from their target sites, while the decrease in gas-phase potency with increasing temperature may similarly be due to heat driving the anaesthetic off these sites.

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ARTICLES

Sr isotopes in interstitial waters of marine sediments from Deep Sea Drilling Project cores

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Sr isotopic compositions of interstitial sea waters have been documented in detail with cores from 37 Deep Sea Drilling Project sites. The ^{87}Sr/^{86}Sr ratios are sensitive indicators of diagenetic reactions involving alteration of volcanic matter dispersed in sediments and recrystallization of carbonates. Effects of basalt alteration reactions, except possibly for hydrothermal signals, and of alteration of continental debris are minimal. Carbonate recrystallization has a significant effect on the geochemical mass balance of Sr but diffusion of unradiogenic strontium from interstitial waters has only a small effect on the ^{87}Sr/^{86}Sr ratio of ocean water.

IN the study of geochemical cycles much recent attention has been given to evaluating differences in the isotopic compositions of the various geochemical reservoirs. ^{87}Sr/^{86}Sr ratios of marine deposits have been reported extensively and have proven to yield useful isotopic indicators of the provenance of marine sediments[1], the involvement of seawater in the geochemistry of hydrothermal sediments[2], in basalt alteration[3] and in mineral formation[4]. In particular, ^{87}Sr/^{86}Sr ratios have been used to evaluate the carbonate record of seawater evolution through time[5–7].

Less attention has been given to measuring the ^{87}Sr/^{86}Sr ratio of sea waters. Because the current oceanic residence time of Sr ($\sim 10^6$ yr) is much longer than the mixing time of the oceans ($\sim 10^3$ yr) it has been generally assumed that the Sr isotopic composition of seawater is the same everywhere[7]. The value for modern ocean water is close to 0.7092.

However, there are two areas in the marine realm where considerable variability in the dissolved ^{87}Sr/^{86}Sr ratio can and does occur. One is in seawater hydrothermal systems[8,9]. The other is in the interstitial waters of marine sediments[10,11]. Sea waters buried with sediments are subject to major compositional changes because they reflect the diagenetic evolution of the sediments and alteration reactions in underlying basalts[12,13]. Earlier measurements[11] of profiles of the Sr isotopic composition of the interstitial waters at two Deep Sea Drilling Project (DSDP) sites showed decreases in ^{87}Sr/^{86}Sr ratio with burial

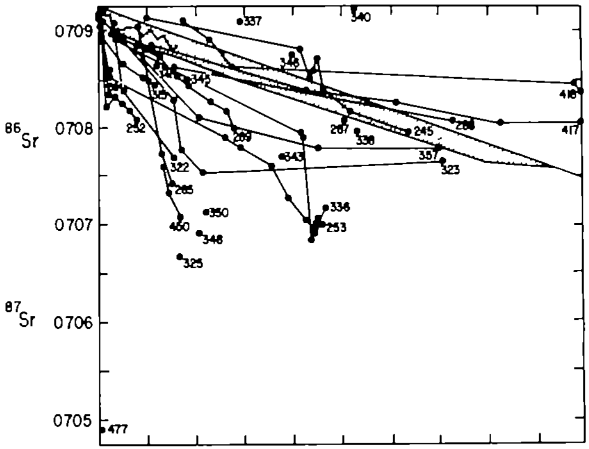

Fig. 1 Plots of ^{87}Sr/^{86}Sr ratios of DSDP interstitial waters versus the age of the sediment sections from which they were extracted. Numbers refer to DSDP sites (Table 1) and the shaded area shows the inferred variation of the ^{87}Sr/^{86}Sr ratio of sea water with time and is defined by: (upper) the linear equation of Hawkesworth and Elderfield[11] and (lower) the fourth order polynomial of Hart and Staudigel[14], the solid line for 0–16 Myr is from ref. 18.

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depth down to ~0.7069. That study prompted a comprehensive programme of measurement of $^{87}Sr/^{86}Sr$ ratios in interstitial waters based on samples from, to date, 37 DSDP sites (Table 1) covering the major oceans and a wide range of sedimentary types. Here we summarize the results, the chief generalizations and the inferences which may be made from the whole data set in terms of processes affecting $^{87}Sr/^{86}Sr$ ratios, temporal changes, and the geochemical mass balance of Sr isotopes in the oceans.

$^{87}Sr/^{86}Sr$ ratios

The Sr isotopic compositions, summarized in Table 1, are plotted in Fig. 1 as a function of the age of the sediment sections from which the interstitial waters were recovered. Examples of the depth profiles are shown in Fig. 2.

Overall, the ratios range from 0.70920 to 0.70904 within 10 m of the sediment surface, values similar to those of contemporaneous sea waters, to a minimum value of 0.70490 in the deepest sample (173 m) obtained from Site 477 in the Gulf of California. With few exceptions the general trend of the data is for $^{87}Sr/^{86}Sr$ ratios to decrease from modern-day values as a function of burial depth and age. However, this decrease does not reflect the changing Sr isotopic compositions of seawater[11,14] because most of the interstitial water values are less radiogenic than contemporaneous sea waters. Such differences cannot be explained purely in terms of diffusive mixing of Sr isotopes within profiles because in many cases the inferred 'age' of the deepest interstitial water sample (using the seawater versus time curve) is greater than the age of the oceanic crust at that site. Even in situations where the age curve for the interstitial waters approximates to that for contemporaneous seawater (for example, Site 245) the Sr isotopic profiles cannot reflect burial of sea waters and no reaction because Sr^{2+} is enriched in the interstitial waters (Fig. 2). Therefore, the Sr isotopic data must reflect geochemical reactions affecting the compositions of the interstitial waters.

Sources of strontium

The $^{87}Sr/^{86}Sr$ ratios in potential source materials responsible for the Sr^{2+} enrichment and isotopic compositions of the interstitial waters are[11]: (1) continental detritus: $^{87}Sr/^{86}Sr \sim 0.716$; (2) biogenic carbonates: $^{87}Sr/^{86}Sr = 0.7092$ to 0.7075 over 0 to 100 Myr, that is the range for contemporaneous seawaters; (3) volcanic materials (basalts and volcanic ash and glass): $^{87}Sr/^{86}Sr = 0.7027$ to ~0.705, the low values for fresh basalts and the high values for materials previously altered by reaction with seawater.

None of the more than 160 interstitial waters analysed have $^{87}Sr/^{86}Sr$ ratios which are significantly higher than the ratio for modern seawater, and few samples have ratios which are more radiogenic than contemporaneous sea waters. This rules out continental detritus as a dominant source of the interstitial Sr^{2+} anomalies at most sites. In contrast, the low $^{87}Sr/^{86}Sr$ ratios of many samples relative to the seawater curve (Fig. 1) show that reactions involving the alteration of volcanic materials must have a major role at many sites.

Geochemical reactions

The chemical and isotopic compositions of the interstitial waters at each site give integrated signals of the combination of diagenetic reactions which, in turn, reflect the mixed lithologies and multiple origins of the sediments. In addition diffusion processes through the interstitial water column will tend to smooth out concentration gradients. Nevertheless, it is possible to identify sites where particular geochemical reactions are dominant.

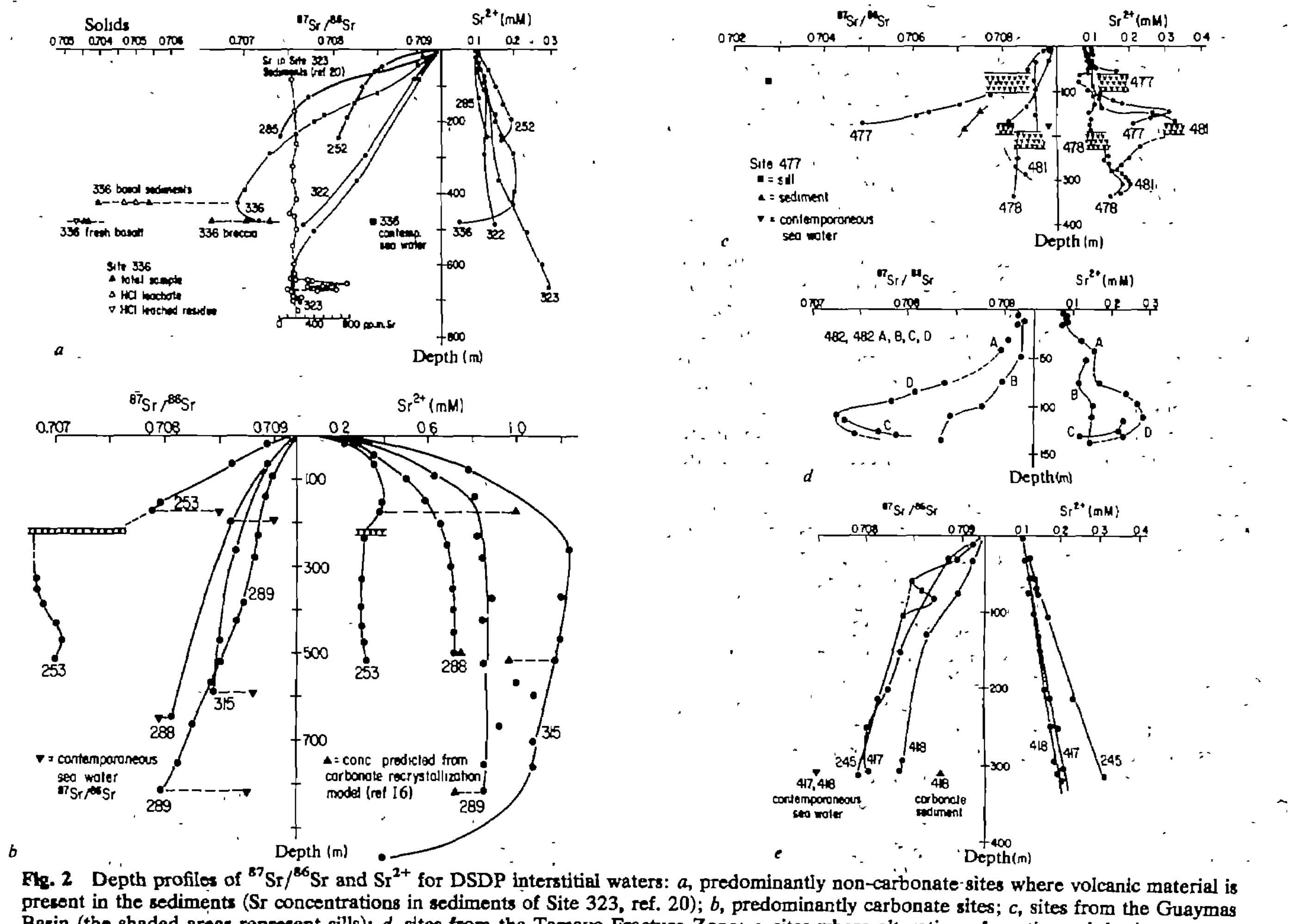

Fig. 2 Depth profiles of $^{87}Sr/^{86}Sr$ and Sr^{2+} for DSDP interstitial waters: *a*, predominantly non-carbonate sites where volcanic material is present in the sediments (Sr concentrations in sediments of Site 323, ref. 20); *b*, predominantly carbonate sites; *c*, sites from the Guaymas Basin (the shaded areas represent sills); *d*, sites from the Tamayo Fracture Zone; *e*, sites where alteration of continental detritus may be occurring.

We now wish to identify the principal types of reaction that can be inferred from the ^{87}Sr/^{86}Sr results when considered alongside the Sr^{2+} and other interstitial water data and the locations and lithologies of the sites.

Alteration of volcanic matter: A major feature reflected in Fig. 1 is that in many sites the ^{87}Sr/^{86}Sr ratios of dissolved strontium are much less radiogenic than those of contemporaneous sea waters. The cause of this phenomenon must be sought in substantial contributions to the dissolved strontium pool by reactions involving volcanic matter.

Figure 2*a* shows profiles from non-carbonate sites containing volcanic matter throughout the sediment column[11,15,16]. Relatively small enrichments of Sr^{2+} in the interstitial waters are accompanied by large decreases in ^{87}Sr/^{86}Sr ratios with depth. At Site 323, the moderate enrichment of Sr^{2+} with depth, ~0.2 mM over ~700 m, was modelled by McDuff and Gieskes[17] in terms of conservative mixing of Sr^{2+} produced from a localized deep source, assumed to be a zone of Danian nanno-chalk and surrounding Sr-rich claystones. The minimum at ~650 m in the Sr isotopic profile defines the deep source as this Sr-rich zone and gives a ^{87}Sr/^{86}Sr ratio of ~0.7075, slightly lower than that for 65-Myr old seawater. The dissolved strontium source, therefore, appears to be derived chiefly from carbonate recrystallization with a small contribution from volcanics. At Site 336, the major Sr^{2+} source is also near-basal sediments. These sediments contain volcanic glass and ash layers are quite common. Comparison of the sediments and associated interstitial waters shows that they are not in Sr isotopic equilibrium.

In all sites presented in Fig. 2*a* it is apparent that at all depths large deviations occur in the ^{87}Sr/^{86}Sr ratio when compared with contemporaneous seawater (see also Fig. 1). The implication of this is that during alteration of volcanic material dispersed in the sediment column extensive exchange of strontium isotope occurs, without a concomitant large effect on the dissolved Sr^{2+} distribution.

The important question arises whether alteration of basalts of Layer 2 of the oceanic crust also contributes to the Sr^{2+} and ^{87}Sr/^{86}Sr signals. Such alteration reactions are held responsible for the often observed large increase in Ca^{2+} and decreases in Mg^{2+}, K^{+} and ^{18}O in the overlying interstitial waters[12,13]. Evidence from dissolved Sr^{2+} profiles (see Fig. 2*a*) indicates reversals in the ^{87}Sr/^{86}Sr and Sr^{2+} gradients where near basement was sampled. Results from the base of Site 336 show that Sr isotopic equilibration of the interstitial water and a basaltic breccia zone has occurred whereby the altered rocks have been severely contaminated by seawater Sr. From the Sr contents and ^{87}Sr/^{86}Sr ratios it seems that the alteration occurred with an effective water–rock ratio of about 20. Thus, although the hydrolysis reaction imparts a Sr isotopic signal to the interstitial water, the resulting ^{87}Sr/^{86}Sr ratios are not strikingly unradiogenic because of the water-dominated exchange reaction. We surmise that no clear basement signal in ^{87}Sr/^{86}Sr is observed as a result of high water–rock ratios, and that the major process leading to the low ^{87}Sr/^{86}Sr ratios in interstitial waters is the alteration of volcanic matter dispersed in the sediment column. This, of course, makes this isotopic ratio particularly useful in studies of sediment diagenesis.

Carbonate recrystallization: Although a volcanic Sr signal is undoubtedly present in many of the profiles, the isotopic data cannot be explained simply in terms of the input of strontium to the interstitial waters by dissolution from an unradiogenic source. This is because the profiles which show the least extreme ^{87}Sr/^{86}Sr gradients show the greatest Sr^{2+} enrichments and vice versa (Fig. 2*b*). The sites showing large Sr^{2+} enrichments (for example, Sites 315, 289, 288, 253) are all carbonate sites. In these sites, interstitial Sr^{2+} concentrations increase to a concentration maximum, up to over 10 times normal seawater levels (Fig. 2*b*) and the ratios of Sr^{2+} and Ca^{2+} concentrations, in excess of those of seawater (that is $>7\times10^{-3}$) are larger than Sr/Ca in basalts, clays and biogenic carbonate ($\sim2\times10^{-3}$). These enrichments are caused by the release of Sr^{2+} during diagenetic recrystallization of inorganic calcite producing Sr-depleted chalks and limestones[18,19,22]. In such cases the interstitial ^{87}Sr/^{86}Sr ratios will remain close to contemporaneous seawater values provided that active recrystallization is occurring so as to minimize the effect of diffusion.

Site 253 indicates a case where a strong volcanic signal is superimposed on the profile expected from pure carbonate recrystallization. Sedimentation rates in the upper 150 m have been relatively low (~4 m Myr^{-1}) and the volcanic sediments below the basaltic sill at 150 m serve as a sink for Sr^{2+} as well as a source of low radiogenic strontium.

Hydrothermal activity: A thermally induced signal in ^{87}Sr/^{86}Sr has been observed in the interstitial waters at sites in regions of high heat flow on young oceanic crust (Fig. 2*c*, *d*). At site 477, located on a large heat flow anomaly (≥20 heat-flow units (h.f.u.)) in the southern rift of the Guaymas Basin, Gulf of California, where the bottom-hole temperature is thought to have exceeded 200 °C there is an extremely strong negative ^{87}Sr/^{86}Sr depth gradient in the interstitial waters down to 0.7049, similar to values for basalt-Sr. The low ^{87}Sr/^{86}Sr ratios are associated with excess ^{3}He values reflecting hydrothermal processes involving basement rocks[21]. At Site 481, in the northern rift of the basin (~4 h.f.u.) the hydrothermal activity must have been associated with the intrusion of a dolerite sill complex which heated the surrounding sediments to as high as ~165 °C (ref. 23) and has now cooled to 45 °C.

Though it is tempting to suggest that the low ^{87}Sr/^{86}Sr ratios in these sites are due to hydrothermal alteration of basalts, it is equally possible that alteration of volcanic debris has caused the ^{87}Sr/^{86}Sr decreases near the sills. This is demonstrated well in Site 482 in the Tamayo Fracture Zone[24], where the high heat flow (12 h.f.u.) has apparently accelerated the rate of alteration of volcanic matter in the sediments producing

Table 1 Summary of Sr isotopic compositions

		^{87}Sr/^{86}Sr		
Site*	Location	No. of samples	Range	Ref.
245	31°32′ S 52°18′ E	8	0.70913–0.70794	11
252	37°3′ S 59°14′ E	7	0.70926–0.70807	†
253	24°53′ S 87°22′ E	9	0.70893–0.70683	†
285	26°49′ S 175°48′ E	4	0.70903–0.70742	†
287	15°55′ S 153°16′ E	1	0.70805	†
288	5°59′ S 161°49′ E	2	0.70861–0.70807	18
289	0°30′ S 158°31′ E	13	0.70918–0.70798	18
315	4°10′ N 158°32′ W	5	0.70904–0.70843	†
322	60°02′ S 79°25′ W	3	0.70892–0.70767	†
323	63°41′ S 97°60′ W	5	0.70897–0.70755	†
324	69°03′ S 98°47′ W	2	0.70890–0.70831	†
325	65°03′ S 73°41′ W	1	0.70667	†
336	63°21′ N 7°47′ W	10	0.70916–0.70694	11
337	64°52′ N 5°21′ W	1	0.70911	†
338	67°47′ N 5°23′ W	1	0.70794	†
339	67°13′ N 6°17′ E	1	0.70899	†
340	67°13′ N 6°18′ E	1	0.70923	†
341	67°20′ N 6°7′ E	1	0.70864	†
342	67°57′ N 4°56′ E	1	0.70898	†
343	68°43′ N 5°46′ E	1	0.70769	†
345	69°50′ N 1°14′ W	1	0.70851	†
346	69°54′ N 8°41′ W	1	0.70875	†
348	68°30′ N 12°28′ W	1	0.70691	†
349	69°13′ N 8°6′ W	1	0.70862	†
350	67°03′ N 8°18′ W	1	0.70713	†
357	30°00′ S 35°34′ W	4	0.70907–0.70777	†
417	25°07′ N 68°03′ W	6	0.70924–0.70802	†
418	25°02′ N 68°04′ W	5	0.70910–0.70836	†
450	18°00′ N 140°48′ W	3	0.70762–0.70708	†
474	22°57′ N 108°59′ W	11	0.70916–0.70765	21
476	23°03′ N 109°06′ W	5	0.70915–0.70866	21
477	27°02′ N 111°24′ W	9	0.70891–0.70490	21
478	27°06′ N 111°31′ W	5	0.70875–0.70822	21
479	27°51′ N 111°38′ W	6	0.70920–0.70894	21
481	27°15′ N 111°31′ W	7	0.70905–0.70816	21
482	22°48′ N 107°60′ W	19	0.70921–0.70733	24
503	4°04′ N 95°38′ W	4	0.70919–0.70903	†

* For information on DSDP sites, see the appropriate volume of *Initial Reports of the Deep Sea Drilling Project* (US Government Printing Office, Washington DC).

† Unpublished results: data report available on request to H.E.

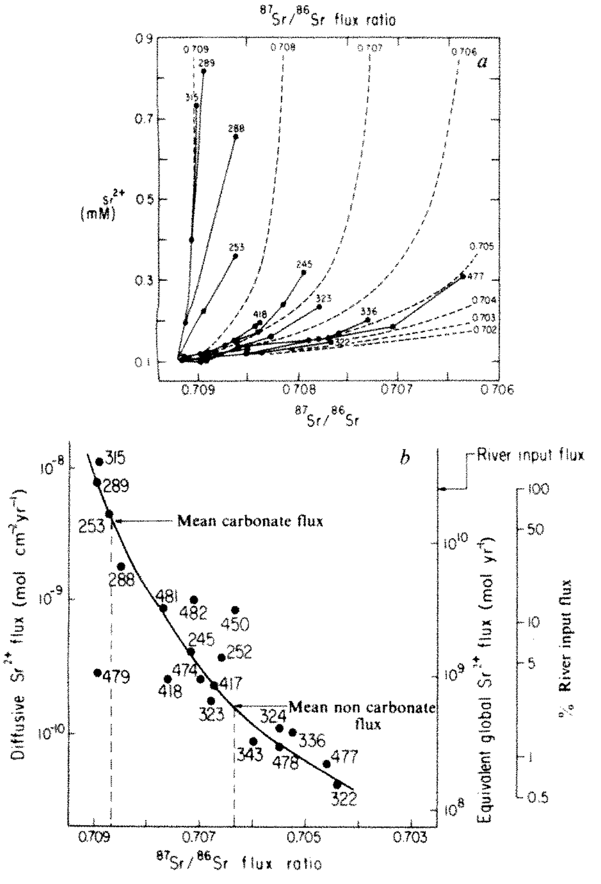

Fig. 3 *a*, Plots of Sr^{2+} against $^{87}Sr/^{86}Sr$ for uppermost sections of interstitial water profiles. Numbers refer to DSDP sites and the curves labelled with $^{87}Sr/^{86}Sr$ ratios are the ratios of fluxes of ^{87}Sr to ^{86}Sr, calculated from: flux (^{87}Sr)/flux (^{86}Sr) = $D(\Delta^{87}Sr/\Delta \text{ depth})/D(\Delta^{86}Sr/\Delta \text{ depth})$, where D is diffusion coefficient. *b*, Diffusive Sr^{2+} flux against $^{87}Sr/^{86}Sr$ flux ratio. Sr^{2+} flux calculated from: $D\Delta Sr^{2+}/\Delta$ depth where $D = 2\times10^{-6}$ cm^2 s^{-1} (ref. 27); flux ratio calculated as above. Numbers refer to DSDP sites. Also shown are average carbonate and non-carbonate fluxes (see text) and river input flux[7]. The equivalent global flux is obtained using the oceanic area of 361×10^{16} cm^2.

pronounced extrema in $^{87}Sr/^{86}Sr$ and dissolved strontium. The isotopic signal is stronger in Holes 482C and D where the thermal influence is thought to have been greater than in Holes 482A and B and, overall, the isotopic changes occur at shallower depths and during earlier diagenesis than at the sites shown in Fig. 2*a* where high temperatures were not experienced. Such $^{87}Sr/^{86}Sr$ changes appear to be associated with temperature-dependent silicification reactions involving the alteration of volcanic matter in the sediments and the diagenesis of opaline silica giving rise to 'diagenetic fronts' in the sediments at depths which relate to the thermal influence at each site[24–26].

Diagenesis of continental debris: Rarely do the $^{87}Sr/^{86}Sr$ profiles show evidence of reaction which may be attributed to material of continental origin having radiogenic Sr isotopic compositions. At Site 245 (Fig. 2*e*) the interstitial waters at ~75 m are distinctly more radiogenic than contemporaneous seawater which may indicate exchange reactions involving continental debris. At Sites 417 and 418 the $^{87}Sr/^{86}Sr$ ratios of the interstitial waters are everywhere significantly higher than for contemporaneous seawaters (Fig. 2*e*) and persist to close to basement into a narrow zone of impure Cretaceous carbonates contaminated with continental detritus which appears to be the main Sr^{2+} source. However, these sites are characterized by very slow sediment accumulation rates (~3 m Myr^{-1}) and diffusive communication with the overlying ocean will exist throughout the pore water profiles. Thus, the high $^{87}Sr/^{86}Sr$ ratios may reflect diffusive mixing of Sr-isotopes. Similar arguments could made for Sites 337 and 340 (Fig. 1). Therefore, no sites yet studied show unequivocal evidence of a Sr-isotopic signal reflecting diagenesis of continental debris.

The above discussion reveals that the major processes affecting the $^{87}Sr/^{86}Sr$ distribution in interstitial waters of deep sea sediments are the alteration of volcanic matter dispersed in the sediments and the recrystallization of carbonates. Even in drill sites consisting almost of pure carbonate sediments a volcanic signal is often superimposed on that expected from carbonate recrystallization processes. Effects of alteration reactions involving basalts of Layer 2, with the possible exception of hydrothermal signals, and of the alteration of continental debris are minimal.

Geochemical mass balance

One of the major aims of the DSDP interstitial water programme has been to evaluate the exchange of chemicals between the oceans and the sediments and basalts of oceanic crust. It has been shown that the diffusive fluxes of the major constituents Ca^{2+}, Mg^{2+} and K^+, as deduced from interstitial waters concentration gradients, do not have a significant role in the geochemical mass balance of the oceans whereas the oxygen isotopic flux may be of importance[12,13].

The Sr^{2+} concentration gradients shown in Fig. 2 are about 0.5–1.0 mM per 100 m for carbonate sites and about 0.02–0.03 mM per 100 m for non-carbonate sites where volcanic matter is undergoing alteration. With an average diffusion coefficient of 2×10^{-6} cm^2 s^{-1} (ref. 27), and neglecting advection, the values are equivalent to integrated global fluxes of the order of $1\text{–}2\times10^{10}$ mol yr^{-1} for carbonate sites and $5\text{–}7\times10^{8}$ mol yr^{-1} for non-carbonate sites, as compared with the river input flux of 2.5×10^{10} mol yr^{-1} (Fig. 3*b*). Assuming that carbonate sediments occupy 25% of the sea floor one calculates an average Sr flux from carbonate sites of ~20% of the river flux. The flux from non-carbonate sites is trivial, globally ~2% of river input.

The effect of these interstitial Sr^{2+} fluxes on the Sr isotopic balance of the oceans is small. This is because the largest interstitial Sr^{2+} gradients (and, hence, the largest fluxes) are associated with smallest $^{87}Sr/^{86}Sr$ depth gradients and vice versa. A combination of Sr^{2+} and $^{87}Sr/^{86}Sr$ data allows the diffusive flux ratios of ^{87}Sr to ^{86}Sr to be defined using the ratios of their concentration gradients versus depth (Fig. 3). Figure 3*b* shows a very consistent pattern for nearly all sites with high values of the $^{87}Sr/^{86}Sr$ flux ratio associated with high Sr^{2+} fluxes and low values of the isotopic flux ratio associated with low total fluxes. The large average Sr^{2+} flux from carbonate sites has an average $^{87}Sr/^{86}Sr$ ratio of ~0.7087, close to the modern seawater ratio as compared with non-carbonate sites which have an average $^{87}Sr/^{86}Sr$ ratio of ~0.7064. However, the latter have a lesser effect on the oceanic Sr isotope budget because the associated Sr^{2+} flux is an order of magnitude smaller. Overall, the effect is small and of minor significance in the oceanic budget of Sr-isotopes[8,9]. This evaluation does not, of course, include the transport by advection of interstitial waters with anomalous $^{87}Sr/^{86}Sr$ ratios. It has been suggested[8,9] that the flux of hydrothermal fluids supplied to the ocean by advection plays a significant role in fixing the $^{87}Sr/^{86}Sr$ ratio of seawater. The advection of interstitial waters with low $^{87}Sr/^{86}Sr$ ratios because of the influence on hydrothermal processes would add to this flux.

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Oxygen isotope evidence for shallow emplacement of Adirondack anorthosite

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Oxygen isotopic analysis of wollastonites from the Willsboro Mine, Adirondack Mountains, New York reveals a 400-ft wide zone of ^{18}O depletion at anorthosite contacts. Values of $\delta^{18}O$ vary more sharply with distance and are lower (to −1.3) than any yet reported for a granulite facies terrain. Exchange with circulating hot meteoric water best explains these results and implies that the anorthosite was emplaced at relatively shallow depths, <10 km, in marked contrast to the depth of granulite facies metamorphism (23 km). These ^{18}O depletions offer the first strong evidence for shallow emplacement of anorthosite within the Grenville Province and suggest that regional metamorphism was a later and tectonically distinct event.

MASSIF-TYPE anorthosite is an important rock type in the Grenville Province and in many other deeply eroded Precambrian terrains of US–Canada. Most of these bodies were metamorphosed in granulite facies conditions that imply burial depths of 20–30 km (refs 1–3). This observation is commonly used as evidence that anorthosite selectively intrudes and solidifies in the lower crust and thus may be a major constituent of a mafic layer below the Conrad seismic discontinuity.

In the Adirondack Mountains, New York and elsewhere petrological evidence has been used to suggest that anorthosite intruded at shallow depths before deep burial and metamorphism. Buddington[4] interpreted the borders of the Adirondack Massif as a chill margin with massive contact skarns and therefore proposed that it intruded shallowly into comparatively cool country rocks. The presence of wollastonite, an index mineral of the sanidinite facies[5,6], supported this view[7]. However, wollastonite has now been recognized in many amphibolite and granulite facies terrains[2,8–11] indicating that the classical assumption that $P_{\text{lithostatic}} = P_{CO_2}$ is not valid for the metamorphism of all marbles[2,11,12]. Thus the petrological evidence is ambiguous, permitting either shallow or deep intrusion[13–15].

We have studied the C–O–H stable isotope geochemistry and phase equilibria of contact skarns in the Adirondacks. While phase equilibria are inconclusive as to depths of emplacement, oxygen isotope studies at the Willsboro Mine, a massive skarn, provide new data that bear on this important problem.

The Willsboro Mine

The Willsboro Mine, an important source of wollastonite since 1940, is located in a 0.25-km thick skarn belt at the contact of the Port Kent–Westport unit of the main Adirondack anorthosite massif. This skarn belt extends over 7 km in the Willsboro quadrangle and may continue an additional 8 km into the Au Sable quadrangle, connecting two other wollastonite deposits at Deerhead and Lewis, New York. The anorthosite forms a structural trough in the region of this belt and shows evidence of extensive magmatic assimilation of metasediment, including common xenoliths of marble and evolved anorthosite compositions[4,16]. Similar features are found on mountain tops in adjacent quadrangles, indicating that originally overlying metasediments were engulfed in the roof zone of the anorthosite body.

The wollastonite ore-rock is distinctively similar throughout these three deposits, consisting of parallel layers of three coarse grained minerals: wollastonite, clinopyroxene, and grandite garnet. Compositional variation is limited and of major-element solid solutions, only Fe/Mg and Fe/Al vary among the pyroxenes and garnets of different layers. At Willsboro two major and many minor layers of ore-rock dip 30–55° NNE with anorthosite forming the footwall of the deposit. Surrounding and between these layers of ore-rock are layers of mixed gneisses including mafic gneiss, amphibolite, and granitic gneiss.

All rocks in the Adirondacks, except a few late dykes, show the effects of a pervasive metamorphism that occurred during the Grenville orogeny. At Willsboro and elsewhere in the north-east Adirondacks, the maximum pressure and temperature of granulite facies equilibration are estimated from many mineral thermometers and barometers to be 7±1 kbar and 700±50 °C (refs 1, 3, 17, 18). U/Pb, Rb/Sr and Nd/Sm ages indicate that metamorphic recrystallization overprinted earlier igneous events[19–21]. Various age estimates suggest that the anorthosite intruded at 1,200 Myr, at least 100 Myr before the peak of metamorphism, but it cannot be resolved whether igneous activity was a precursor of metamorphism with intruding magmas contributing heat to raise the geotherm, or whether intrusion and metamorphism represent two discrete, unrelated events.

The Willsboro skarn belt gives clear evidence of a metasomatic origin. The wollastonite ore-rock consistently contains three phases and five components for 4 degrees of freedom, and it is commonly reported to be totally devoid of any calcite or quartz[22]. We have located a few thin layers containing calcite at Willsboro (see Table 1), but these amount to only a few centimetres out of thousands of metres of drill core that were examined. If wollastonite formed through isochemical metamorphism (with only fluid components added or subtracted) through the reaction:

$$\text{Calcite} + \text{Quartz} = \text{Wollastonite} + CO_2 \qquad (1)$$

the entire original limestone unit would have had to contain an impossibly perfect molar ratio of 1 calcite to 1 quartz. Thus large amounts of material were either removed or added in solution, indicating that massive fluid circulation was involved in the ore genesis.

Stable isotope results

Analysis of mineral separates from 18 samples of drill core from a 700 ft traverse of the Willsboro Mine (at 7,600 W, mine coordinates) shows that unusually low values of $\delta^{18}O$ (2.9 to -1.3) are restricted to localities within 400 ft of the anorthosite contact. Taylor[23] reported a similar low value of -0.9 for one sample of wollastonite from Willsboro. These low values form a steep-sided trough (Fig. 1) with sharp gradients to higher $\delta^{18}O$ in the gabbroic anorthosite of the footwall and in the mafic gneisses of the hanging wall. The magnitude of this trough is normalized in Fig. 1 by a line connecting wollastonite analyses or, in the case of rocks not containing wollastonite, through the value of a fictive wollastonite, calculated assuming equilibrium isotopic fractionations at 700°C (refs 24, 25).

Other wollastonite samples from the main layers of ore-rock at Willsboro as well as from Lewis and Deerhead (Table 1) have similarly low $\delta^{18}O$ values (3.1 to -1.2) suggesting that the entire 15-km skarn belt is ^{18}O-depleted near the anorthosite contact. We are making further analyses to test this hypothesis, as it would indicate that the ^{18}O-depleting event affected many cubic kilometres of rock.

Three coexisting minerals were analysed from a sample of Willsboro ore-rock (82-W-1) to test that equilibrium isotopic values[24,25] have been preserved from the peak of regional metamorphism (Table 1). These mineral analyses approximate the values that are expected at 700 °C considering that the fractionations and chemical compositions are not well known. The large ^{18}O anomaly cannot have formed by retrograde exchange with post-metamorphic fluids as such an exchange would differentially affect the different minerals.

The gradients in this isotopic trough are the sharpest yet reported for non-carbonate-bearing rocks from any granulite facies terrain. In drill hole DDH 50, $\delta^{18}O$ values vary by 8.0‰ in 14 ft between ore-rock and amphibolite and by 6.0‰ in 3 ft within ore-rock. Additional sampling and analysis will elucidate the details of these gradients. In this respect the apparently parallel layers of the Willsboro deposit cause probelms. In spite of extensive drilling and mining it is not now known to what extent these gradients are due to tectonic juxtaposition through faulting, recumbent folding, or deformation. Faulting, in particular, could cause gradients in $\delta^{18}O$, and the coarse regional metamorphic recrystallization of the deposit must be considered, as it could effectively conceal evidence of such movements if they were premetamorphic. We have not seen any textural evidence of faulting in the zones of high $\delta^{18}O$ gradient, nor are indications of faulting reported by previous workers. Thus if tectonic processes are responsible for these gradients, they operated during an earlier event before peak metamorphic recrystallization, and these gradients were then preserved through the granulite facies metamorphism.

Seven samples of amphibolite were also anlysed for hydrogen isotopic composition. Unfortunately the measured δD values of -86 to -68 fall in a range commonly observed in igneous, metamorphic and sedimentary rocks and are not indicative of the origin of fluids involved in the genesis of this deposit.

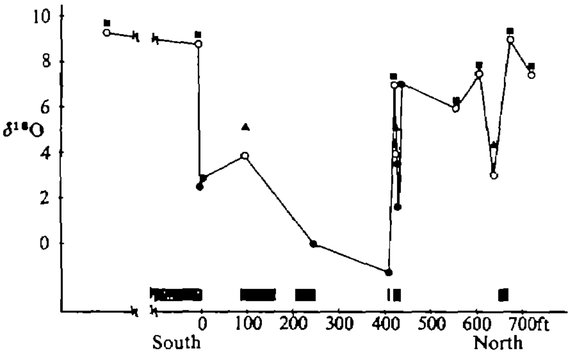

Fig. 1 Values of $\delta^{18}O$ for minerals from a 700-ft traverse of the anorthosite contact with wollastonite skarn at the Willsboro Mine. The 400-ft wide trough of ^{18}O depletion includes the lowest $\delta^{18}O$ (-1.3) and the sharpest $\delta^{18}O$ gradients known from a granulite facies terrain. Plagioclase in the hanging-wall anorthosite (south) is isotopically similar to other Adirondack anorthosites. ●, Wollastonite (measured); ○, wollastonite (calculated); ■, plagioclase; ▲, calcite; solid shading, wollastonite ore-rock; and hatching, anorthosite.

Cause of the ^{18}O depletion

Large ^{18}O depletions occur at igneous contacts by only two processes: metamorphic volatilization and exchange with hot meteoric water. Estimates of the effects of volatization, however, indicate that they are insufficient to account either for the extreme magnitude of ^{18}O depletion (up to -20‰) or the extreme size (cubic kilometres) of the Willsboro skarn belt.

Unusually low $\delta^{18}O$ values have also been reported from granulites of widely separated portions of the Strangways Range, Australia (1.8‰ for hypersthene, 2.8‰ for cordierite)[26]. Additional mechanisms for ^{18}O depletion have been proposed for these rocks including premetamorphic diagenesis and high temperature metamorphic exchange with deep-seated mafic reservoirs. These processes cannot have been important at Willsboro as the ^{18}O anomaly is restricted to the skarn belt and is not seen in any of 85 regionally distributed marble samples that have normal values of $\delta^{18}O$ (ref. 27).

Volatilization can be considered as the slow release of CO_2 due to the reactions such as (1). The magnitude of ^{18}O depletion is then largest for reaction in shallow terrains, in spite of the pressure independence of equilibrium isotope fractionations, because temperatures of reaction will be lowest at low pressures, resulting in higher enrichments of ^{18}O in the escaping CO_2. We have made equilbrium calculations at 300–600 °C based on the stoichiometry of well-known, idealized reactions for the complete decarbonation of a siliceous marble. These calculations suggest that the extremes of ^{18}O depletion for regional and contact metamorphism are approximately 5 and 7‰, respectively. These ^{18}O depeletions would be even less if decarbonation is not complete, if non-reacting minerals are present in the rock, if H_2O or CH_4 dilute the metamorphic CO_2, or if degassing is not controlled by Raleigh distillation[28] with equilibrium fractionations. If isotopic fractionations are not equilibrium fractionations, then the magnitude of ^{18}O depletion due to volatilization will again be reduced. Experiments on the rapid, non-equilibrium, thermal decomposition of dolomite show that the evolved CO_2 is only slightly enriched in ^{18}O relative to dolomite[29].

Results from the Mottled Zone, Israel, should represent an extreme case of volatilization depletion. In response to heat from naturally burning organic matter, calcite completely reacted with silicates at depths of <100 m, and the measured ^{18}O depletions are generally <7‰ (ref. 30). Similar results are observed for low pressure contact metamorphism at Marble Canyon, Texas, where a difference of 9‰ (normalized to wollastonite) was found between adjacent zones of calcite+wollastonite and spurrite[31]. Some ^{18}O depletion in these areas could have been caused by hot circulating groundwaters, and thus these values may be viewed as upper limits. Numerous other contact aureoles have been shown to be less affected by volatilization[31–35].

In the Adirondacks analyses of 85 carbonates average $\delta^{18}O = 20$ (ref. 27). This is in keeping with the average $\delta^{18}O$ value of 21 for worldwide unmetamorphosed carbonates of the same 1,200 Myr age[36]. In contrast the Willsboro wollastonite is depleted by >20 ‰. This ^{18}O depletion is 3–5 times larger, depending on depth of metamorphism, than is found at other documented localities or than our calculations indicate is possible through volatilization.

Table 1 Values of $\delta^{18}O$, δD and $\delta^{13}C$ for mineral separates from the Willsboro Skarn Belt

Sample no.	Description	$\delta^{18}O$	$\delta^{13}C$	δD
AUS 78-1	Plagioclase megacryst from anorthosite, Lewis Quarry	9.7		
AUS 78-4	Wollastonite from ore-rock, Lewis Quarry	−1.2		
81-AUS-1	Wollastonite from ore-rock, Deerhead Quarry	1.2		
W 2-3b	Calcite from marble 2.5 km ENE of Willsboro Mine	21.9	1.1	
DDH W 50-1′	Wollastonite from ore-rock in main ore zone, DDH 50 at 1-ft level, Willsboro Mine	0		
DDH W 50-163′	Wollastonite from ore rock, 3-ft layer within amphibolite	−1.3		
DDH W 50-177′	Plagioclase from amphibolite	6.7		
	Hornblende from amphibolite			−69.6
DDH W 50-178′	Calcite from 2-cm thick layer of calcite+clinopyroxene+scapolite	5.5	−1.4	
DDH W 50-181′	Wollastonite from ore-rock, 19-ft layer within amphibolite	3.5		
DDH W 50-183′	Wollastonite from ore-rock	1.0		
DDH W 50-186′	Wollastonite from ore-rock	7.0		
DDH W 50-216′	Biotite from amphibolite			−75.4
DDH W 50-287′	Biotite from amphibolite			−73.3
DDH W 50-307′	Plagioclase from amphibolite	6.3		
	hornblende	4.8		
DDH W 50-351′	Biotite from amphibolite			−68.5
DDH W 50-357′	Plagioclase from amphibolite	7.9		
DDH W 50-391′	Calcite from 10-cm thick layer of calcite+garnet+clinopyroxene	4.4	−2.2	
DDH W 50-425′	Plagioclase from amphibolite	9.3		
	whole rock			−86.2
DDH W 50-475′	Plagioclase from amphibolite	7.8		
	biotite from amphibolite			−78.0
W 77-100	Plagioclase from foot-wall mafic gneiss in outcrop at no. 4 portal at Willsboro Mine	9.8		
W 77-103	Plagioclase from hanging wall amphibolite in outcrop at no. 2 portal at Willsboro mine	8.6		
	hornblende	5.8		−86.0
DDH W 81-3-5′	Calcite from 5-cm thick layer of calcite+clinopyroxene+garnet	5.1	−3.6	
DDH W 81-3-88′	Wollastonite from 1.3-m thick layer of ore-rock	2.9		
DDh W 81-3-91′	Wollastonite from same layer as −88′	2.5		
DDH W 81-3-101′	Plagioclase phenocryst from gabbroic anorthosite	9.3		
DDH W 81-7-1′	Wollastonite from ore-rock in foot wall 1,200 ft ESE of DDH W 81-3	3.1		
DDH W 81-9-94′	Plagioclase phenocryst from gabbroic anorthosite 200 ft WNW of DDH W 81-3	7.8		
81-W-21	Plagioclase phenocryst from anorthosite 2 km E of the Willsboro Mine	9.1		
82-W-1	Coexisting minerals from ore rock, Willsboro Mine			
	wollastonite	−0.7		
	clinopyroxene	0.3		
	garnet	−0.5		

If the ^{18}O depletion at Willsboro resulted from volatilzation of CO_2 then a complimentary ^{13}C depletion should be seen. Carbonates from contact aureoles that have undergone variable amounts of volatilization often show a direct correlation between $\delta^{18}O$ and $\delta^{13}C$ for this reason[30–35]. The ratio of ^{13}C depletion to ^{18}O depletion is controlled by the relevant CO_2-mineral fractionations and the mechanism of volatilization, but ^{13}C depletion is always at least one-third that of ^{18}O. Three calcites from thin lenses (2–10 cm thick) within the Willsboro ore-zone have an average $\delta^{13}C = -2.4$. This is 2.5‰ less than the average of 75 Adirondack calcites (0.1)[27] indicating that the magnitude of ^{18}O depletion at Willsboro is ≤7.5‰, in accord with our estimate based on calculations and other field studies.

Exchange with heated meteoric waters is a well documented process, causing massive depletions of up to 15‰ to $\delta^{18}O$ values below −10 at the margins of cooling plutons where convective fluid flow facilitates high water-to-rock ratios[37]. Exchange with meteoric water is thus the most probable cause of ^{18}O depletion, especially given the association of wollastonite with the roof zone of the anorthosite massif.

It is common for hot circulating meteoric waters to lower the ^{18}O content of the solidified margins of a pluton as well as the surrounding country rock. In the Adirondacks, the $\delta^{18}O$ values of plagioclase in anorthosite (8.9–11.2) are generally 3‰ higher than anorthosites and gabbros elsewhere. However, five unusually garnet-rich anorthosites have low $\delta^{18}O = 4.3$ to 5.7 (ref. 23). Regardless of whether the general ^{18}O enrichment occurred during the metamorphic[23] or magmatic[38] event, some additional process is necessary to explain these garnet-rich anorthosites that are lower in ^{18}O than normal anorthosite. These samples are from near the contacts of the anorthosite and have been interpreted as resulting from metasomatic interaction with metasedimentary wall rock[4,16]. It is reasonable to suggest that these anorthosite samples have also interacted with meteoric water.

Shallow intrusion of anorthosite

The involvement of large amounts of meteoric water in the skarn formation at Willsboro would require a depth of anorthosite emplacement much less than the 23 km inferred for granulite facies metamorphism. This is because meteoric waters circulate at hydrostatic pressures (P_H) and cannot penetrate to depths of high grade metamorphism, where porosity is extremely low and fluids are at lithostatic pressures ($\sim 3P_H$). The maximum depths to which groundwater circulates are estimated[39,40] at 7–10 km. Many documented examples of ^{18}O exchange with meteoric waters occur at the margins of such shallowly emplaced plutons[41], and we propose that the Adirondack massif likewise intruded at shallow depths of < 10 km.

Shallow anorthosite intrusion is supported by the presence of contact aureoles at two anorthosite massifs from outside of the Grenville Province. At Nain coexisting orthopyroxene+olivine+quartz limits pressure[3,13] to 3.2±0.2 kbar, and at Laramie coexisting pigeonite+hedenbergite+fayalite limits pressure[42] to 1.5–2.0 kbar. These bodies lie to the north-west of the Grenville Front and do not show Grenville metamorphic overprints. It is thus likely that North American anorthosites intruded at all levels in the crust before the Grenville orogeny and that subsequently those nearest the continental margin were buried at the time of metamorphism. Such rapid burial is supported in the Adirondacks by whole-rock Rb/Sr ages of 1,263 ± 25 Myr for surface eruption of ash-flow tuffs that were subsequently metamorphosed (1,120 Myr) at 20 km depth to leuco-gneiss[21]. The common inference may thus be incorrect and the close association of anorthosite with granulite facies metamorphism seems to have no relevance to actual emplacement depths.

We believe that these Adirondack results are best explained by the following events. Anorthositic magma intruded to shallow depths at ~1,200 Myr. Metasomatism and skarn formation took place during solidification and cooling in the roof-zone with the involvment of large amounts of low-δ^{18}O meteoric water. The anorthosite and skarn were subsequently buried to 23 km and metamorphosed at ~1,100 at low f_{CO_2}.

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Evidence for the direct involvement of DNA replication origin in synthesis of late SV40 RNA

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Analysis of the late simian virus 40 (SV40) transcription initiation patterns of viable and non-viable mutants and a DNA replication-negative mutant in permissive monkey cells and/or in Xenopus laevis *oocytes suggests a direct role for the origin of DNA replication in the activation of late transcription.*

THE SV40 genome contains two transcription units. The early unit is functional in permissive cells throughout the infection cycle and in SV40-transformed cells. The minimal promoter elements and more remote regulatory functions have been characterized in detail[1–4]. The late transcription unit, however, is actively transcribed only in permissive cells during the late phase of the infection cycle. Late mRNA is not synthesized in significant quantities in transformed cells, in lytically infected cells in the presence of inhibitors of DNA synthesis[5,6], or in cells infected at the non-permissive temperature with temperature-sensitive (ts) A mutants[6,7]. Furthermore, microinjection experiments with form I and replicative intermediate DNA into chemically blocked permissive cells or non-permissive cells, also suggested a role of DNA replication in late mRNA synthesis[8,9]. However, other data obtained from studies with transcription complexes[10,11], early mRNA[12], or mRNA from infected non-permissive cells[13], led to the rejection of the idea of a special role for DNA replication in the activation of the late SV40 promoter. These studies suggest that the large amount of late mRNA is caused by a marked increase in the gene copy number. The much smaller amount of early RNA observed at late times after infection may be explained by the autoregulatory function of large T antigen[14,15].

We attempted to resolve this controversy and to give a better definition in molecular terms of the requirements for synthesis of late SV40 mRNA. We therefore analysed the RNA produced by several deletion mutants in the putative late promoter region[16] (D. G. *et al.*, in preparation) and a DNA replication-negative mutant in infected CV1 cells and/or in *X. laevis* oocytes. Extensive deletions in the late initiation region were found to have a very limited effect on downstream initiation points, but a deletion of four base pairs (bp) that had been shown to prevent DNA replication[17] caused a marked inhibition of late RNA synthesis even at some downstream initiation points over 300 nucleotides away.

5′-Termini of wild-type and deletion mutant late mRNAs in CV1 cells

The 5′-termini of late lytic SV40 RNAs are strikingly heterogeneous[18–20]. The positions of these 5′-termini have been mapped by reverse transcription[18] and by direct analysis[19,21].

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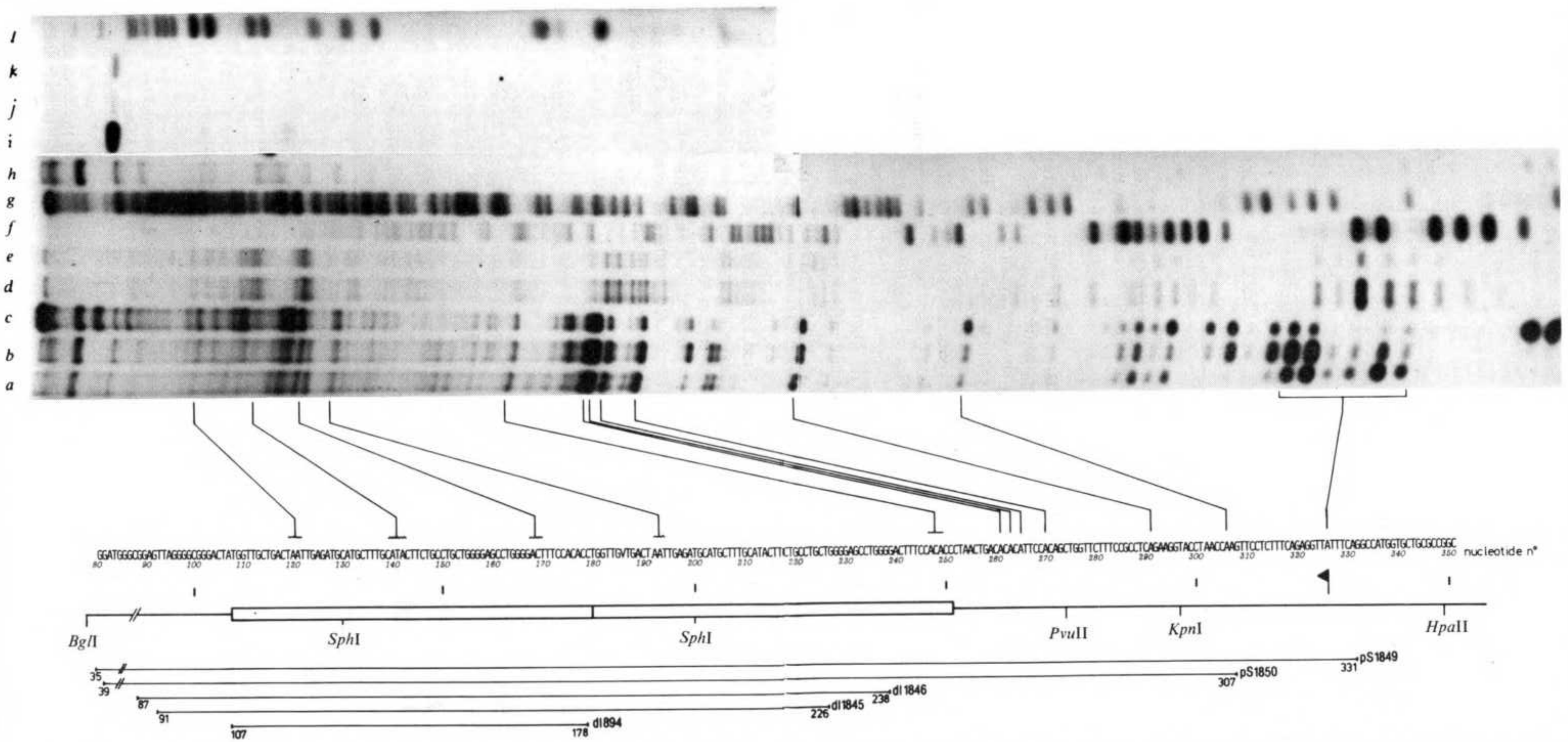
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Fig. 1 Mapping of 5′ termini of SV40 late mRNA with S_1 nuclease and exonuclease VII. The upper part of the figure shows a 12% polyacrylamide–8 M urea gel of wild-type (wt) SV40 late mRNA 5′-termini. (Migration in all the figures is from left to right.) 1 μg of poly $(A)^+$ RNA isolated from SV40-infected CV1 cells 48 h after infection as described elsewhere[19], was hybridized to a single-stranded (SS), 5′-end-labelled *Hpa*II–*Mbo*II DNA probe, and digested with exonuclease VII at increasing concentrations of NaCl (*a*, 50 mM; *b*, 200 mM; *c*, 300 mM) or with S_1 nuclease (*d*, *e*) as described elsewhere[22,45] (*d* and *e* are identical experiments carried out separately). The 5′-labelled restriction fragments in this and all following experiments were strand-separated on a 5% polyacrylamide gel as described[46], and an aliquot of the isolated single strand was co-precipitated with each RNA preparation. After hybridization in 15 μl buffer (40 mM PIPES *p*H 6.4, 400 mM NaCl, 1 mM EDTA) for a minimum of 4 h, the hybrids were digested with S_1 nuclease or exonuclease VII. Lanes *f* (T+C) and *g* (A+G) show a Maxam–Gilbert[46] DNA sequence ladder. Lane *h* is a control digest of the probe. Lanes *i*–*k* are S_1 nuclease digests in conditions identical to those of *d* and *e* with strands separated from the SV40 *Hind* F RI fragment. As this fragment is localized in the body of the VP1 gene, the late coding strand is entirely protected against S_1 nuclease digestion (lane *i*); the other strand is not protected (lane *j*). Lane *k* shows the late coding strand digested in the absence of RNA and lane *l* is a chain length marker. This experiment (lanes *i*–*l*) was carried out on a 20×40 cm polyacrylamide sequencing gel, while lanes *a*–*h* were fractionated on a 30×90 cm gel. The lower part of the figure shows the nucleotide sequence of the region where the late SV40 mRNAs initiate. The initiation points as deduced from a comparison of lanes *a*–*d* with lanes *f* and *g* (and considering data in the literature; see text) are indicated. The identification of the exact 5′ ends of the four longest bands (wavy lines) is less accurate than for the others; they were assigned from a longer run (results not shown). Some characteristic restriction sites and the 72-bp repeats are also shown. The flag indicates the major SV40 wt initiation of late mRNA at position L325. The positions of the deletion mutants are indicated at the bottom; the deletions are identified by the nucleotide numbers (the numbering system is as described previously[47,48]). The deletion mutants were constructed as follows: pBR325 was deleted for the tetracycline gene by partial *Hae*II digestion, which produced the plasmid pBGF1 (D. G. *et al.*, in preparation). Wild-type SV40 and d1 894 DNA were digested with *Eco*RI and ligated in the unique *Eco*RI site of the chloramphenicol resistance gene. After opening at the *Sph*I site in the cloned SV40 DNA, treatment with nuclease *Bal*31, followed by re-ligation, produced the series of deletions which were characterized by nucleotide sequence analysis.

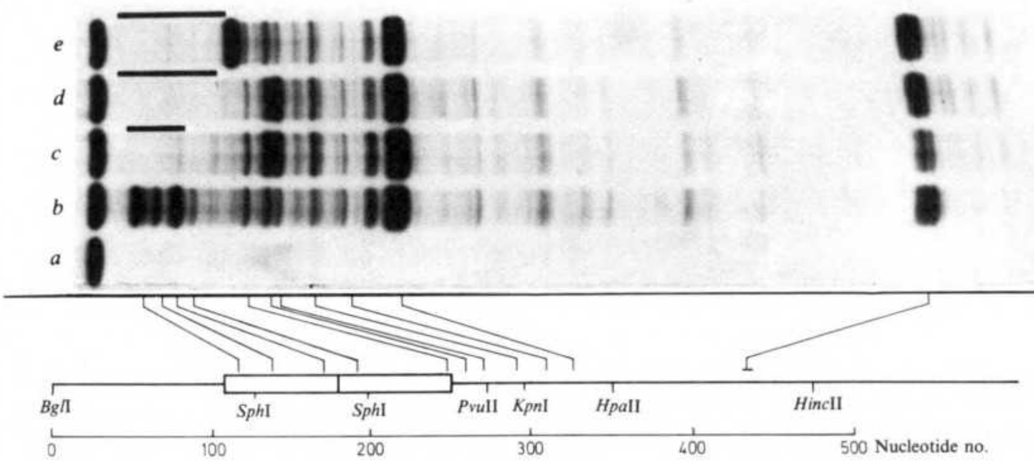


Fig. 2 Exonuclease VII mapping of the 5′-ends of wt- and viable mutant-induced late poly(A)$^+$ mRNA extracted from CV1 cells. Cytoplasmic poly(A)$^+$ mRNA of wt and mutant viruses extracted at 48 h post-infection was hybridized against the single-stranded [5′-^{32}P]-labelled *Bgl*I–*Hin*dII (position 472) fragment and the RNA–DNA hybrid digested with exonuclease VII as described in Fig. 1 legend. *a*, 1 μg poly(A)$^+$ mRNA from uninfected CV1 cells; *b*, 1 μg poly(A)$^+$ mRNA of wt-infected CV1 cells; *c*, 0.5 μg poly(A)$^+$ mRNA from dl 894-infected cells; *d*, 1.0 μg poly(A)$^+$ mRNA from dl 1845-infected cells; *e*, 1.0 μg poly(A)$^+$ mRNA from dl 1846-infected cells. The size and position of the deletion is shown on the polyacrylamide–urea gel by a solid line (see also diagram in Fig. 1). The lower part of the figure is a schematic representation of the wt initiation points and major restriction enzyme sites.

Previously we have shown that the physical 5′ cap structures correspond to genuine initiation points for transcription[16]. For the present analysis of the late mRNAs, we used the S_1 nuclease and/or exonuclease VII mapping technique with single-stranded probes [5′-^{32}P]-labelled at the *Hpa*II (Fig. 1) or *Hin*dII sites (Fig. 2). An S_1 nuclease and an exonuclease VII 5′-end analysis of wild-type mRNA are shown in Fig. 1. As in a high resolution gel, three to five bands are formed per 5′-end after an S_1 digestion, the pattern obtained is rather complex and is not very well suited for mapping at the exact nucleotide level especially in regions where several 5′-ends occur close together (see lanes *d* and *e* of Fig. 1). The pattern produced by exonuclease VII mapping is similar to the S_1 nuclease pattern but is more discrete. Comparison of the S_1 pattern and the direct 5′-end analysis data[19,21] with other data in the literature[22], shows that the major band of the S_1 nuclease-digested DNA is two nucleotides longer than its RNA counterpart. This phenomenon is even more pronounced with exonuclease VII where in most cases the DNA is four to five nucleotides longer than the RNA to which it was hybridized (possibly the enzyme is inhibited by the positive charge on the cap structure). The patterns of the initiation points which can be deduced from the gel analyses reported here, are very similar to those obtained from reverse transcription mapping[18,20]. Note also that some of the late 5′ initiations start in the 72-bp repeats (Fig. 1).

To test the hypothesis that the remarkable heterogeneity of initiation points is caused by tandemly repeated promoter elements[16], a series of deletion mutants was constructed by nuclease *Bal*31 digestion of *Sph*I-opened SV40 DNA cloned in plasmid pBGF (D.G. *et al.*, in preparation; see also Fig. 1). Analysis of the late mRNA obtained from CV1 cells infected with the largest viable mutants, dl 1845 and dl 1846, and with the natural deletion mutant, dl 894, is shown in Fig. 2. The analysis involved exonuclease VII digestion with a DNA probe labelled at the *Hin*dII site at position 472 (similar results have also been obtained with an *Hpa*II probe labelled at position 348). Four major conclusions arose from these experiments: (1) The analyses showed that the initiation patterns downstream from the deleted sequences were almost unchanged when compared with the wild-type 5′-termini, suggesting that

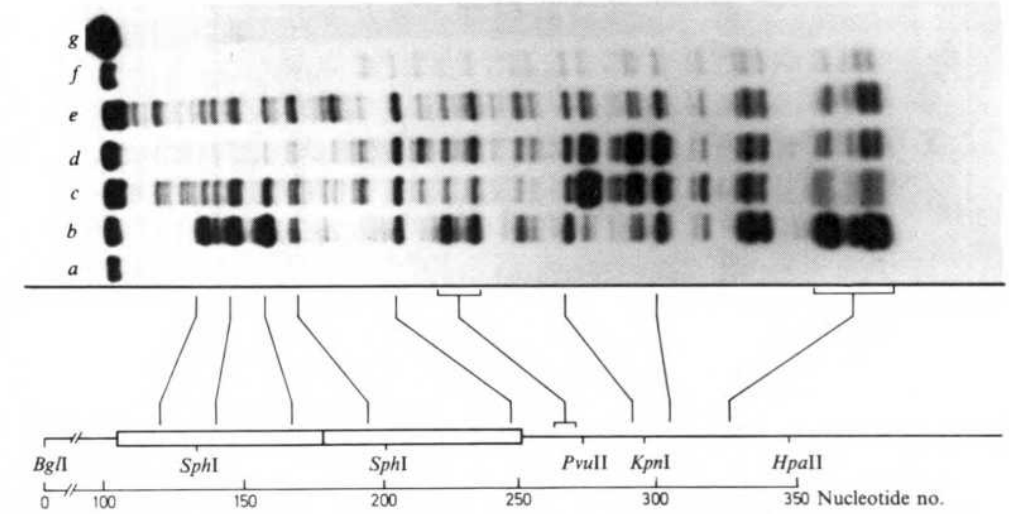


Fig. 3 Mapping of the 5′ ends of SV40-specific RNA synthesized in *X. laevis* oocyte nuclei injected with wt templates. Stage VI oocytes were injected with 10 ng sucrose gradient-purified DNA aiming for the nucleus [23,49] and incubated at 22 °C for 6 h. The nucleus and cytoplasm were separated after heating the oocyte at 75 °C for 3 min, followed by making an incision and manual removal of the nucleus. The nuclear RNA was isolated by hot phenol extraction and the preparation was further digested with proteinase K-treated DNase I[50] (twice for 15 min at 37 °C with 1 μg per nucleus), phenolized with hot phenol and co-precipitated with the isolated [5′-^{32}P]-labelled single-stranded probe. Hybridization and exonuclease VII digestion were as described in Fig. 1 legend. *a*, RNA from uninjected oocytes; *b*, reference pattern of poly(A)$^+$ mRNA from CV1-infected cells (same as lane *b* in Fig. 2); *c*–*e*, RNA from oocytes injected with: *c*, viral wt DNA; *d*, pSCLO$_4$ DNA; *e*, pSMboC DNA; *f*, not relevant to this experiment; *g*, undigested single-stranded probe. Each gel analysis was carried out with three oocyte RNA equivalents. The wt templates pSCLO$_4$ and pSMboC plasmids were constructed as follows: pSCLO$_4$ contains the SV40 *Hin*dII + *Hin*dIII fragments C and L (568 nucleotides) and was made by addition of a *Bam* linker to the *Hpa*I site at position L501 and cloning between the *Hin*dIII and *Bam*HI sites of pBR322. pSMboC contains the SV40 *Mbo*II fragment C (710 nucleotides) which was blunt-ended, *Bam* linkers were added and the fragment was cloned in the *Bam*HI site of pBR322. The SV40 formation in both pSCLO$_4$ and pSMboC spans the region of DNA replication and transcription initiation.

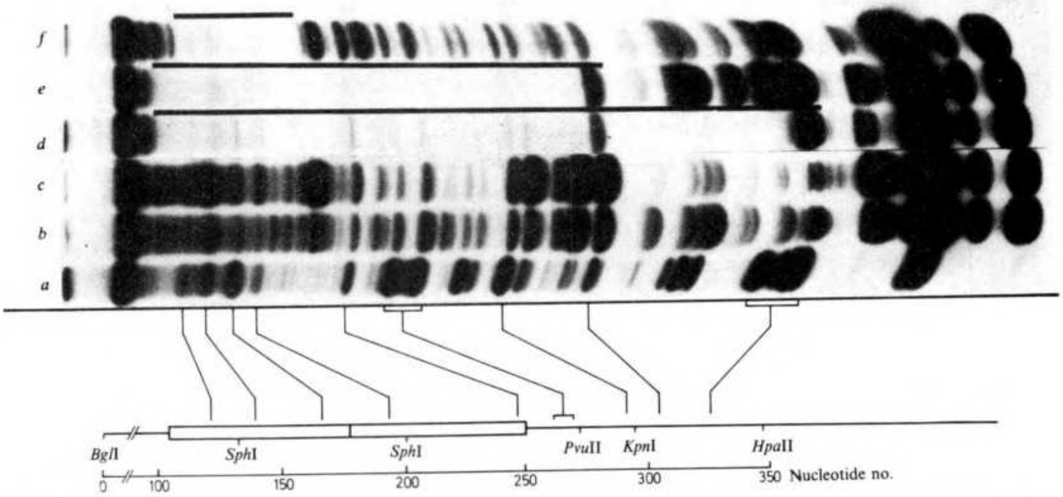


Fig. 4 5′-End analysis of oocyte nuclear RNA synthesized on SV40 wt and deletion mutant templates. The experimental procedure was as described in Fig. 3 legend. *a*, CV1 wt poly(A)$^+$ mRNA reference pattern (same as for Fig. 2*b*); *b*, injection with wt viral DNA, 4 h incubation; *c*, injection with wt viral DNA, 22 h incubation; *d*, injection with mutant pS1849 DNA; *e*, injection with mutant pS1850 DNA; *f*, injection with mutant dl 1846 DNA. The position of the deletions is shown by a heavy horizontal line above the corresponding lanes (see also the map of the mutants at the bottom of Fig. 1). The darker band in lanes *d* and *e* near the *Kpn*I site is a background band, as no initiation sites can be present at this position.

the deletions in the regions immediately upstream have little function in the positioning and synthesis level of the remaining RNA species. (2) As these viable deletions also delete major parts of the 72-bp 'enhancer' sequences, which are required for early gene expression[2], it seems that the most crucial elements of the 'enhancer' sequences are localized at the right part of the repeat and/or that other regulatory mechanisms (for example, T-antigen autorepression) back-up for a decreased enhancer function. (3) The dl 1846 pattern, the largest viable deletion in this region, shows a new strong band located at the end of the deletion, which could represent an artificial new initiation point formed by the combination of nucleotides around the deletion; alternatively, it could be derived from RNAs initiated in foreign DNA inserted at the position of the deletion. Indeed, it was found (D.G. *et al.*, in preparation), that the viral DNA collected after CV1 infection, especially that from the mutant dl 1846, showed some heterogeneity in the *Hin*d C fragment (the mutant dl 1845 was found to be more stable in this respect). To avoid this complication, late SV40 transcription was also analysed in *X. laevis* oocytes (see below). (4) There is also a predominant initiation point around position 433. This site probably corrresponds to the initiation sites mapped by Piatak *et al.*[20] at positions 428–481. Again, the position of this initiation point was not shifted by extensive upstream deletions.

During the construction of the deletion mutants, several non-viable mutants having more extensive deletions in the presumed promoter region were also isolated (for example, pS1849 and pS1850). The transcriptional behaviour of these and the other mutants was examined in *X. laevis* oocytes.

Initiation of wild-type and mutant late SV40 transcripts in nuclei of *X. laevis* oocytes

Xenopus oocytes have been shown to synthesize late[23,24] and early[25] SV40-encoded products. Faithful promoter-specific initiation, however, has not yet been demonstrated. Figure 3 shows the 5′-end analysis of SV40-specific RNA synthesized in *X. laevis* oocytes injected with various wild-type templates (Fig. 3*c–e*) compared with *bona fide* late mRNA from CV1 cells (Fig. 3*b*). It is evident that the wild-type viral RNA initiation pattern in oocytes (Fig. 3*c*) closely resembles the pattern found in CV1 cells (Fig. 3*b*). There are some small differences, mainly quantitative, in the relative strength of transcription initiation, rather than qualitative differences in the pattern of transcription. At later times (22 h), these differences increase but the basic pattern remains the same (Fig. 4*c*). The results indicate that oocyte nuclei provide a reliable and faithful system for the study of SV40-directed transcription at least at short times after injection (6–7 h). Injection with the SV40 fragments *Hin*d C+L (position E67–L501; for numbering see Fig. 1 legend) or *Mbo*II C (position E236–L474) containing the expression control signals cloned in a pBR322 derivative, again produced the typical late transcription pattern (Fig. 3*d* and *e*, respectively). Therefore, it may be concluded that the fidelity of the transcription of the SV40 sequences is not appreciably affected by neighbouring plasmid sequences. In addition, it is clear from the results obtained with these plasmids that information for large T antigen is neither needed for efficient late transcription in oocytes nor involved in positioning of the late mRNA start sites. This situation resembles the *in vitro* transcription initiation pattern of early SV40 RNA[15] which is similar to an *in vivo* late-early pattern, although it was found that late time initiation patterns of '*in vivo*' early mRNA do require the presence of a functional T antigen[26]. Injection of the non-viable mutant DNA, pS1850 and pS1849 (see Figs 1, 4), which lack almost the entire late mRNA initiation region, showed that here again the deleted sequences apparently have no effect on the downstream initiation points. Therefore, the results obtained with deletion mutants in CV1 cells described here and by others[19,20], and those obtained with extensive deletion mutants in injected oocytes, indicate that unlike the normal system of a classical promoter-driven transcription, the control elements for SV40 late transcription are not localized in the regions immediately upstream (or downstream) from the mRNA initiation site. As discussed above, various data in the literature suggest that DNA replication is linked to the activation of late transcription. To test the possibility of a direct involvement of the SV40 DNA replication region in late transcription, we injected the DNA replication-negative mutant 8-4[17,27] into *X. laevis* oocytes.

Transcription of a DNA replication-negative mutant in *Xenopus* oocytes

The 5′-end of the late SV40-specific RNA synthesized in *X. laevis* oocytes injected with the plasmid pSCLO$_4$ (containing the SV40 control region from E67 to L501) and with the DNA replication-negative mutant 8-4 DNA, is shown in Fig. 5 (lanes *c* and *d*, respectively). Clearly the amount of late SV40 RNA isolated from the nucleus of mutant 8-4-injected oocytes is greatly reduced, although the remaining mRNAs are still faithfully initiated. The possibility of some artefact such as faulty nuclear injection with the mutant DNA is excluded by two different controls. First, in the experiments shown, wild-type and mutant DNAs were mixed with an equal amount of DNA from the plasmid p(325)-gHFIF-4, which contains the gene for human fibroblast interferon[28], and the incubation medium of the injected oocytes was assayed for interferon. The results show even better interferon synthesis by the oocytes injected

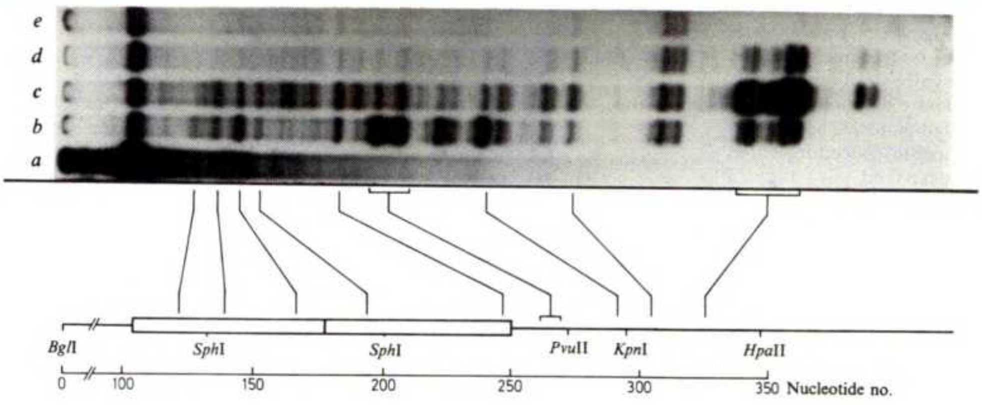


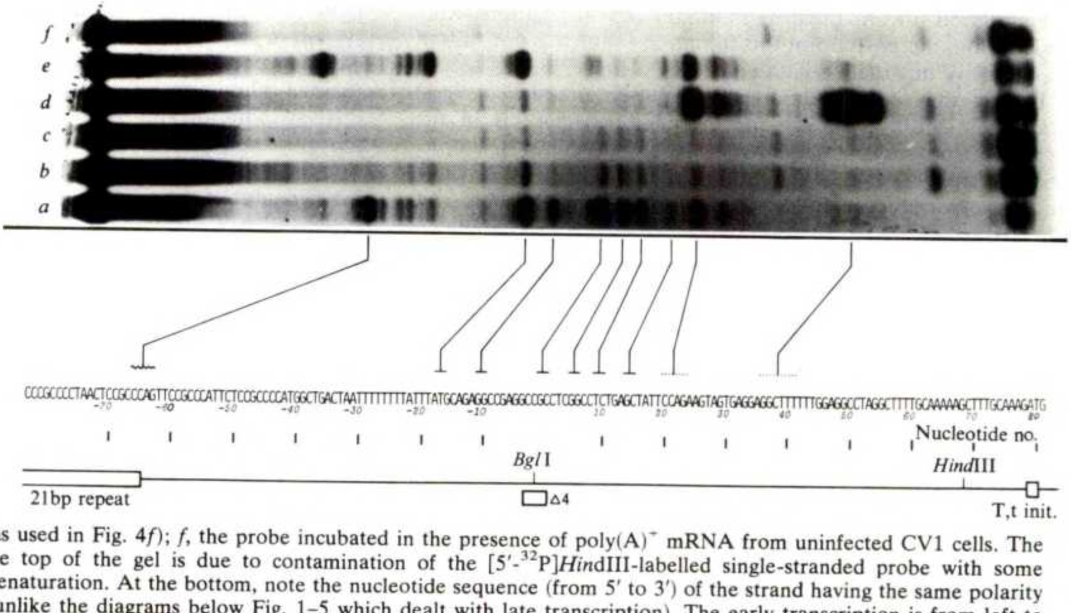
Fig. 5 5′-End analysis of SV40-specific late RNA synthesized in *X. laevis* oocyte nuclei injected with a plasmid containing an SV40 DNA replication-negative mutant. *a*, Undigested single-stranded probe; *b*, poly(A)$^+$ mRNA of wt-infected CV1 cells, reference pattern; *c*, injection with the plasmid pSCLO$_4$ containing a functional wt SV40 DNA replication region (same as used in Fig. 3*d*) plus p(325)-gHFIF-4 DNA as an internal control; *d*, injection with the DNA replication-negative mutant 8-4 (see below) plus p(325)-gHFIF-4 DNA as an internal control; *e*, nuclear RNA from oocytes injected with plasmid p(325)-gHFIF-4, which encodes human fibroblast interferon[28] (internal control for lanes *c* and *d*). The mutant 8-4 was constructed by Gluzman *et al.*[17] by directed mutagenesis of pMK16/SV40 DNA at the *Bgl*I site. The deletion of four nucleotides at the *Bgl*I site does not influence the synthesis of T antigen (and hence the major type of early mRNA synthesis), but prevents DNA replication and production of V antigen and infectious virus[17].

with mutant than by those injected with wild-type DNA. The validity of the experiment was further corroborated by data obtained from the analyses of the early SV40 RNAs synthesized in the same oocyte nuclei (see Fig. 6), which showed that the amount of early RNA from the DNA replication-negative mutant is even higher than the amount of RNA from the wild-type-injected oocytes (the higher early transcription may be coincidental or may be due to the absence of competition for transcription factors by an inactive late region). Densitometer tracings of the gels and visual interpretation indicate that there is a reduction of at least 10-fold in late SV40 RNA synthesis by the DNA-replication-negative mutant compared with transcription from the wild-type template. The possibility of gene copy number amplification (despite the non-permissivity of this system[29]) of the wild-type pSCLO$_4$ DNA was analysed by quantitation of the DNA by dot hybridization and by analysing the methylation pattern of the injected DNA. Although by dot hybridization we could not exclude a slight increase (1.3–1.9-fold) in the amount of nuclear plasmid DNA 7 h after injection, the methylation pattern of the DNA as analysed by a Southern blot of *Mbo*I and *Sau*3A digests was unchanged during the incubation, indicating the absence of even a single round of DNA replication (data not shown). In addition to the quantitative aspect of late transcription by the origin-defective mutant, there is also a difference in the initiation pattern of early transcription. It was observed that the initiation positions of early RNA which appear only at late times after infection[26,30] (after the onset of DNA replication) are used considerably less by the origin-defective mutant than by the wild-type DNA (Fig. 6*b*, *d*). Instead, the more rightward initiation points around nucleotide E40 are more pronounced. We propose that in addition to the classical polymerase II promoter for early transcription, there are heterogeneous early transcripts which are formed as a result of the activation of the DNA replication origin.

Discussion

RNA polymerase II promoters have been well characterized in different (*in vitro* and *in vivo*) systems by the construction of deletion mutants in critical regions. In this manner the function of the TATA box was elucidated[31–33], evidence for the function of a −70 signal (the CAAT box) was obtained from studies *in vivo* (including oocytes)[34–36], and a more upstream 'enhancing' function was found[2,37].

The late transcription unit of SV40 is unlike most cellular or viral transcription systems because it is only actively expressed


f
e
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b
a
CCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTTGCAAAGATG
-70
-60
-50
-40
-30
-20
-10
10
20
30
40
50
60
70
80
Nucleotide no.
BglI
HindIII
21bp repeat
Δ4
T,t init.

Fig. 6 5′-End analysis of SV40-specific early RNA synthesized in *X. laevis* nuclei. *a*, Poly-(A)$^+$ mRNA from wt-infected CV1 cells, reference pattern (48 h after infection); *b*, *c*, injection with plasmid pSCLO$_4$ containing a wt DNA replication region (same RNA as used in Fig. 5*c* for analysis of late SV40-specific RNA); *d*, injection containing the plasmid containing the SV40 DNA replication-negative mutant 8-4 (same RNA as used in Fig. 5*d* for analysis of late SV40-specific RNA); *e*, injection with pS1846 DNA (same RNA as used in Fig. 4*f*); *f*, the probe incubated in the presence of poly(A)$^+$ mRNA from uninfected CV1 cells. The dark full-size band near the top of the gel is due to contamination of the [5′-^{32}P]*Hin*dIII-labelled single-stranded probe with some complementary strand and renaturation. At the bottom, note the nucleotide sequence (from 5′ to 3′) of the strand having the same polarity as the early SV40 mRNA (unlike the diagrams below Fig. 1–5 which dealt with late transcription). The early transcription is from left to right. The initiation points were identified relative to a chemical degradation pattern[46] run on another gel. The RNA initiation points do not correspond exactly with those given by Ghosh and Lebowitz[26], but the differences may be due to the different methods used and these precise assignments do not affect our main conclusions. The leftmost initiation point is mapped less accurately (wavy line). Also indicated on the diagram is one of the 21-bp repeats, the 4-nucleotide deletion at the *Bgl*1 site in the mutant 8-4, and the start codon for synthesis of small t- and large T-antigen synthesis. (T, t init.).

in permissive cells, and because of its high degree of transcriptional start site heterogeneity. Neither our own results for CV1 cells described here, nor the results of others with SV40 (ref. 38 and W. Schaffner, personal communication) or with polyoma virus[39], have indicated a function of the aforementioned classical promoter elements for late viral transcription. Therefore, an analysis of larger (and hence non-viable) deletions in the region upstream of the major transcription initiations was required. We therefore explored the possibility of studying the late SV40 transcription system in nuclei of *Xenopus* oocytes and found that it was faithful. As in the CV1 system, however, we were unable to find any evidence of classical polymerase II promoter elements by analysis of a series of appropriate deletion mutants. Instead, we found a clear coincidence between the DNA replication function and an essential function required for late RNA synthesis located within four nucleotides at the *Bgl*I site. This unexpected result can be explained by assuming either that the region of DNA replication is directly required for late mRNA synthesis, or that the overlapping of functions is purely incidental. The latter explanation would imply the presence of a remote control element, as yet unidentified, that would be involved in transcriptional initiation at sites some of which are over 300 nucleotides downstream. We therefore consider it more likely that the mutation 8-4 at the *Bgl*I site[17] largely abolishes late transcription due to an impaired DNA replication function.

The 'special' structure present in SV40 DNA, which has an active origin of DNA replication and which functions as a novel type of polymerase II promoter, may be either a 'DNA replication eye', a D-loop structure, a region where special DNA replication proteins bind, or a special conformation of the origin DNA formed in the nuclear environment. This 'special' structure is presumed not to be formed or to be formed to a considerably lesser extent by the *Bgl*I deletion mutant, 8-4.

Hay and De Pamphilis[40] recently proposed that SV40 DNA replication starts with the synthesis of DNA having the same polarity as early mRNA, whereby a single-stranded region is exposed on the retrograde template which is the same strand as is transcribed for late RNA synthesis. As it was generally found that class II RNA polymerases prefer denatured DNA as template[41], this single-stranded region on the retrograde arm may allow the formation of a stable complex with the RNA polymerase which then begins to travel along the template and initiates RNA synthesis at many but non-random positions, giving rise to late SV40 mRNA transcripts.

It may be argued that *X. laevis* nuclei do not constitute a permissive system for SV40 DNA replication but as mentioned above, the special structure formed by the origin of DNA replication and implicated in late SV40 transcription does not necessarily even involve incipient DNA strand synthesis. It may be formed either by properties intrinsic to the DNA sequence itself or by interaction with nuclear components whose properties are conserved even between CV1 cells and *X. laevis* oocytes. In permissive monkey cells, T antigen may be responsible for the progression of the special structure to an active DNA replication complex. In non-permissive cells, such as mouse or rat cells, T antigen which binds to the replication origin region[14] may be responsible for shut-off of all late transcription. Of course, additional factors may be involved in distinguishing a permissive system from a non-permissive system (for example, factors needed for expression of the V antigens). Note, however, that Subramani *et al.*[42] have observed expression in non-permissive cells of a dihydrofolate reductase gene cloned in the late region of SV40.

Ghosh and Lebowitz[26] have shown that there is an increase in 5'-terminal heterogeneity of early SV40 mRNA at late times in infection. Similar observations had been reported for polyoma virus early mRNA[30]. If the heterogeneity of the late RNA initiations were coupled to the formation of a special DNA structure in the origin region, as we propose, then the increase in heterogeneity of early start points could be produced by a similar mechanism—that is, direct activation of a second type of transcription system by the DNA replication region.

Thus, we believe we have demonstrated a novel type of gene regulation. RNA polymerase II can either initiate transcription on classical promoters or it can initiate by a mechanism driven by a DNA replication origin. Considering that many genes are regulated in function of the cell cycle, it certainly seems meaningful to couple the expression of several genes to DNA replication. Indeed, the phenomenon of regulation of late SV40 gene expression by DNA replication may also have a role in the expression of some cellular genes, such as the regulation of histone synthesis as observed in yeast[43], Chinese hamster ovary cells[40] and hamster fibroblast cells[44].

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Are there black holes in quasars?

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There are two main theoretical models for the central engine of quasars and other active galactic nuclei: the black hole model and the spinar model. Observations of the time scales of flux variations can rule out the black hole model and are therefore extremely important. The widely accepted argument of Elliot and Shapiro[1] states that the maximal luminosity of the nucleus is given by the Eddington limit, $L_{Edd} = 10^{38}$ $(M/M_\odot)$ erg s^{-1}, and the minimal time scale for periodicity is given by t_{min} = (gravitational radius)/(velocity of light) = 10^{-5} $(M/M_\odot)$ s. Therefore, the observed time scale of variations t, and the luminosity L must obey $\log t > \log L - 43$. Variations on a time scale shorter than that would rule out the black hole model. We point out here that this statement is wrong in view of recent developments in the accretion disk theory: much shorter periodicities are still consistent with the black hole model.

One of the most important new results of the theory of accretion disks is that the observed flux of geometrically thick disks can be formally consistent with luminosities up to 100 times (but probably not much more) greater than the Eddington luminosity[2-5]. This is not only due to genuine super-Eddington luminosities of the disks, but mainly because the radiation is highly beamed in a long and narrow vortex along the axis of rotation. This high flux in the beam, when multiplied by the surface of the whole celestial sphere, can give formally a luminosity of the order of $100 L_{Edd}$, when the intrinsic luminosity of the nuclei is only a few Eddington luminosities. The possibility that the radiation of quasars and other active galactic nuclei, especially BL objects, is highly beamed was suggested a few years ago by Blandford and Rees[6]. The agreement of this hypothesis with quasar statistics is very good[7]. Figure 1 (taken from ref. 5) shows how highly the radiation is beamed. The observed flux is formally consistent with a luminosity

$$L = \lambda L_{Edd} = 1.26 \times 10^{38} \lambda \left(\frac{M}{M_\odot}\right) \quad \text{erg s}^{-1} \qquad (1)$$

where the dimensionless, model-dependent, parameter λ obeys the condition $\lambda \leqslant 10^2$.

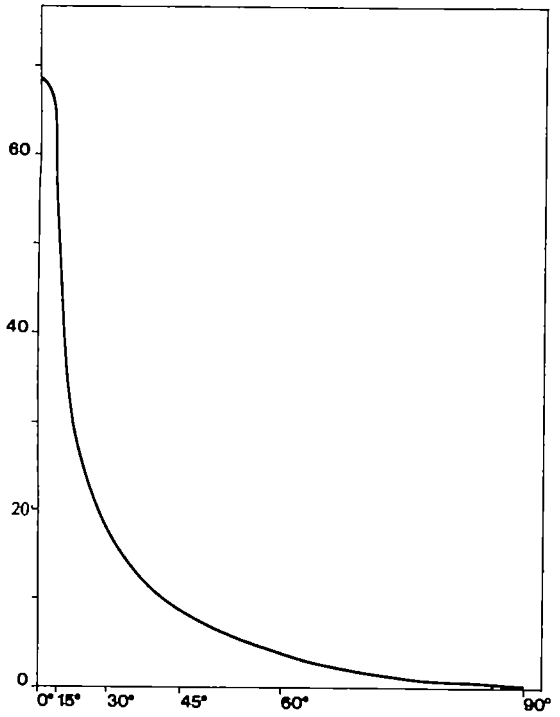

Fig. 1 Beam of radiation emerging from the funnel of a thick accretion disk. The luminosity L formally obtained from the observed flux (by assuming an isotropic radiation field) depends on the angle θ which line of sight makes with the axis of the beam. Luminosities substantially greater than L_{Edd} are possible.

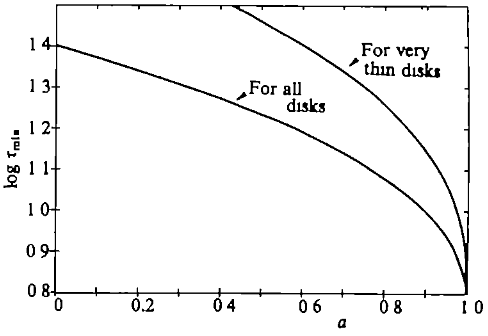

Fig. 2 Log τ_{min} plotted against the Kerr parameter a. Although the condition $\log \tau_{max} > 0.8$ cannot distinguish between thin and thick disks, one must remember that if $\lambda \gg 1$, the thin disks are ruled out.

Another important recent result of the theory of accretion disks is that the innermost edge of the disk does not necessarily coincide with the marginally stable orbit, $r = r_{ms}$, but can be much closer to the hole: as close, in fact, as the marginally bound orbit, $r = r_{mb}$. Frequency of circular orbital motion gives a good estimate of a minimal possible periodicity of flux generated at a given point on the surface of the disk[8]

$$t_{min} = \tau \frac{r_G}{c} = 0.98 \times 10^{-5} \tau \left(\frac{M}{M_\odot}\right) \quad \text{s} \qquad (2)$$

with the dimensionless parameter $\tau \equiv \pi(r_*^{3/3} + a)$ depending on the location of the region which provides the time variation, $r = 1.48 \times 10^5 r_*(M/M_\odot)$ cm, and on the dimensionless Kerr parameter a which characterizes the rotation of the black hole: when $a = 0$ the hole does not rotate, and when $a = 1$ the hole rotates at the maximum possible rate. The locations of the horizon, marginally bound, and marginally stable orbits for the non-rotating black hole are given by $r_* = 2$, $r_* = 4$, and $r_* = 6$ respectively; for the hole rotating at the maximum speed all

Table 1 Estimated values of a_{min} for seven observational points shown in Fig. 3

						a_{min}		
Ref.	Type	Object	z	$\log L$	$\log t$	$\lambda = 10$	$\lambda = 30$	$\lambda = 100$
1	Q	3C454.3	0.860	46.1	3.0	0.90	0	0
1	Q	3C345	0.594	46.2	3.4	0.33	0	0
1	BL	BL Lac	0.07	45.5	2.3	0.98	0	0
9	BL	1308	0.996	47.5	4.5	0.78	0	0
9	BL	0235	0.582	48.2	4.7	*	0.78	0
9	Q	3C446	1.403	48.1	4.6	*	0.78	0
10	BL	OJ287	0.306	47.0	2.97	*	*	0.78

BL, BL Lacerte; Q, quasar; * black hole model ruled out.

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three locations are given by $r_* = 1$. Therefore, the parameter τ must obey $\tau > \tau_{\min} = 2\pi$, or $\log \tau_{\min} = 0.8$ for an $a = 1$ hole and $\tau > \tau_{\min} = 8\pi$ or $\log \tau_{\min} = 1.4$ for an $a = 0$ hole.

The function $\tau_{\min}(a) = \pi[(r_*)^{3/2}_{\mathrm{mb}} + a] = \pi\{[2 - a + a(1 - a)^{1/2}]^{3/2} + a\}$ is shown in Fig. 2.

From equations (1) and (2) we deduce the corrected form of the basic Elliot–Shapiro formula

$$\log t_{\min} = \log L - 43.1 + \log(\tau/\lambda) \qquad (3)$$

If the source of radiation is undergoing relativistic motion then, as pointed out by Blandford and Rees[6], the observed time variation can be even shorter. This, however, cannot be applied to periodic variations observed over many periods as these must be connected with disk rotation. Knowing from observation the values of $t_{\min}$ and L one can find from equation (3) the value of $\log(\tau/\lambda)_{\mathrm{obs}}$. Assuming that $\lambda = 10^2$ is an upper limit for luminosity one can find the upper limit for $\log \tau$, equal to $\log \tau_{\max} = \log(\tau/\lambda)_{\mathrm{obs}} + 2$. If $\log \tau_{\max} < 0.8$ then one can say that observations exclude the possibility of the black hole model. In the case when $0.8 < \log \tau_{\max} < 1.4$ non-rotating black holes are ruled out. Observations are still consistent with rotating black holes for which the Kerr parameter a is bigger than the minimal one, $a_{\min}$, given by the solution of the equation

$$a_{\min}: \quad \log \tau_{\max} = \log \tau_{\min}(a) \qquad (4)$$

Figure 3 shows observational points taken from refs 1, 9 and 10 for seven objects which are closest to the original Elliot–Shapiro restriction (heavy line). The observations of OJ287 by Valtaoja *et al.* which suggest the most extremal flux and the most rapid rotation of the black hole are very recent ones and must still be confirmed. The five thin lines give the shortest possible $t_{\min}$ (that is for $\log \tau = 0.8$) in the case when $\lambda = 1$, 3, 10, 30 and $\lambda = 100$. In Table 1 we present estimations of $a_{\min}$ for these seven points. All the observational points are consistent with the black hole model. Figure 3 indicates that thick, but not thin, accretion disks can explain observations. In some cases observations show that the central black hole must rotate. More detailed analysis of the greater body of observational data is now in progress and the results will be published elsewhere.

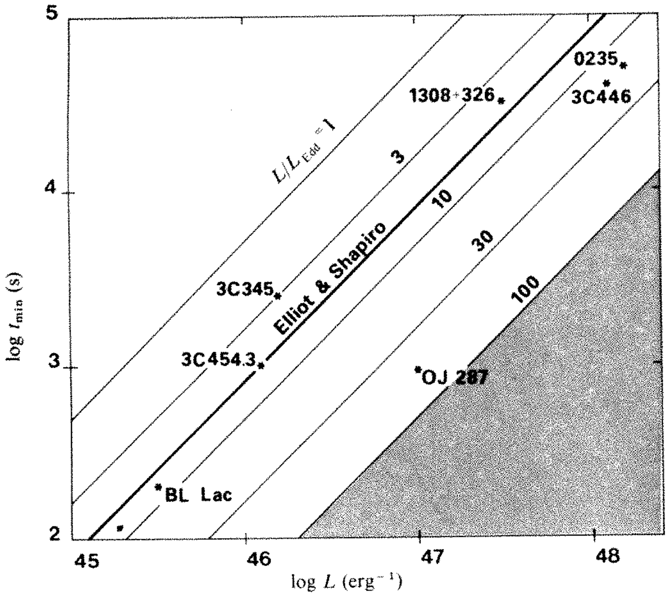

Fig. 3 Seven active galactic nuclei on the log L versus log t plane. The heavy line corresponds to the original Elliot–Shapiro relation ($\lambda = 1 = \tau$). Three thin lines give absolute lower limits for $t_{\min}$ in the cases $\lambda = 1$, 3, 10, 30, 100 ($\log \tau = 0.8$ is assumed on these lines). For almost all the objects $\lambda \gg 1$. This rules out thin accretion disks. Observational points in the dark region would contradict the black hole model.

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$H_2CN^+ \cdot nN_2$ clustering formation and the atmosphere of Titan

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Measurements performed by Voyager 1 have revealed a dense atmosphere around Titan mainly composed of N_2 with some CH_4 and other minor components, including hydrocarbons and organic–nitrogen molecules[1–4]. Based on Voyager and some laboratory data, it is reasonable to assume that other complex organic–nitrogen molecules are formed in Titan's atmosphere[5–7]. Three-body association reactions are probably involved in the synthesis of such compounds[5,7,8]. It has been suggested[5] that the clustering of H_2CN^+ with N_2 or CH_4 could have an important role in these reaction mechanisms. Recently,[8] we observed the $H_2CN^+ \cdot N_2$ cluster ion in a N_2–CH_4 mixture irradiated by α particles. Here we report the results of laboratory measurements of equilibrium constants for the clustering of N_2 with H_2CN^+ leading to $H_2CN^+ \cdot nN_2$ ($n = 1$–6) formation, which should help in understanding the ion–molecule chemistry in the atmosphere of Titan.

The chemical complexity of Titan's atmosphere detected in the course of the Voyager 1 encounter has been accepted to be a result of the coupled chemistry of ion–molecule and neutral reactions[4,5,7,9]. In the lower atmosphere of Titan, where ionization results from cosmic-ray bombardment, lower temperatures and higher neutral densities are suitable conditions for three-body ion–molecule association reactions. The temperature and pressure in the region of maximum cosmic-ray activated chemistry are ~150–160 K and ~20 mbar (ref. 5).

The ion H_2CN^+ probably has a major role in the chemistry of Titan's atmosphere: it can be associated with N_2, CH_4 and C_2H_2 molecules leading possibly to formation of compounds such as CH_3CN, C_2H_3CN ... through three-body ion–molecule reactions, while it is assumed to be partially responsible (with $N(^4S)$ and $N(^2D)$) for HCN formation[10]. Although Voyager 1 did not directly observe any evidence of the presence of the H_2CN^+ ion, electron and ion measurements[11] suggest the presence of an ion with a mass of the order of 28 AMU, which is believed[11] to be H_2CN^+. Recently[12], this ion has been tentatively identified in the Earth's stratosphere: in fact, some of the chemical processes involved in the formation of prebiotic molecules in the atmosphere of Titan are probably similar to those which could have acted in the primitive Earth's atmosphere.

To gain some insight into the ion chemistry on Titan, we carried out a simulation experiment[8] in temperature, pressure and atmosphere composition conditions consistent with the interpretation of the Voyager 1 data. In this, a gas mixture of

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N_2 and CH_4 (~100:1–5) simulated Titan's atmospheric composition while ionization resulted from α particle (^{241}Am, 5.4 MeV, 40μCi cm^{-2}) bombardment. The irradiated gas bleeds through a leak into an evacuated electrode chamber. There the ions carried by the gas are captured by the electric fields while the gas is pumped away. The ions are focused, accelerated and then subjected to mass analysis and electron multiplier detection. The temperature (10–300 K) is carefully controlled and can be varied by means of a cryostat. The experiments were carried out in the pressure and temperature ranges 20–80 torr and 134–293 K respectively. As in our previous experiment, ^{15}N or CD_4 were used to identify the ions studied.

The ion intensities change with temperature in ways that suggest that they are formed by three-body reversible reactions, and thus

$$H_2CN^+\cdot(n-1)N_2+2N_2 \rightleftarrows H_2CN^+\cdot nN_2+N_2, \qquad n=1\text{–}6 \qquad (1)$$

The experimental results are summarized in Fig. 2 where the van't Hoff plots of the equilibrium constants are shown for the three-body association reactions (1). H_2CN^+ is produced by reactions starting with N^+ or $N_3{}^+$ ions and CH_4 (refs 5, 8, 10) (Fig. 1). The equilibrium constants, K_n are deduced from the observed ion current ratios, $I(H_2CN^+\cdot nN_2)/I(H_2CN^+\cdot(n-1)N_2)$, and from the N_2 pressure by the relation

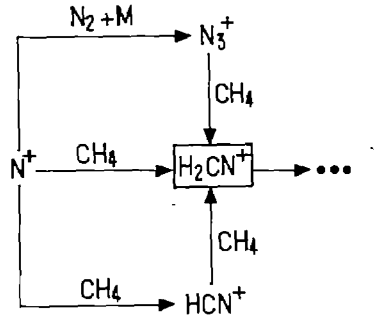

Fig. 1 N^+ ion-molecule reactions with CH_4 and N_2 leading to production of H_2CN^+.

$$K_n = I_n/I_{n-1}P_{N_2} \qquad (2)$$

Corrections were applied for variations in the mass spectrometer sensitivity with respect to the selected mass.

The corrected equilibrium constants are independent of the N_2 pressure, as assumed by relation (2) over the pressure range studied (20–80 torr). Also, within the experimental accuracy, no significant effects were observed on adding different proportions of CH_4 (~1–5%) nor when different voltages were applied in the electrode chamber. This is the experimental evidence of thermodynamic equilibrium.

Thermodynamic properties (Table 1) for the association reactions (1) were derived from the least-squares-fitted linear curves of Fig. 2 using the relation

$$K = \exp(\Delta S^\circ/R - \Delta H^\circ/RT)$$

where R is the perfect gas constant and it is assumed that variation in entropy, ΔS°, is small in the range of studied temperatures.

The data contained in Fig. 2 and Table 1 allow the relative concentrations of the ion clusters to be determined over a wide range of temperatures and pressures. An example of such equilibrium distribution is given in Fig. 3, for the N_2 pressure of 40 torr. This plot, which shows a characteristic behaviour of the reversible reaction (1), was obtained by the following procedure: a set of equilibrium constants was calculated by substituting the desired constant temperature T in the straight-line relationships (Table 1) $\log K_n = [A\times10^3)/T]-B$. The concentration of H_2CN^+ was then set equal to unity. The relative concentrations of the species $H_2CN^+\cdot nN_2$ were obtained using the equation $\log[H_2CN^+\cdot nN_2] = \log K_1 + \log K_2 + \ldots + \log K_n + n \log P_{N_2}$. By setting the sum of the concentrations as 100, the relative concentrations are obtained in per cent of the total.

Table 1 Thermodynamic values for the reactions $H_2CN^+\cdot(n-1)N_2+2N_2 \rightleftarrows H_2CN^+\cdot nN_2+N_2$

n	$-\Delta H^\circ$ (kcal mol^{-1})	$-\Delta S^\circ$ (cal mol^{-1} K^{-1})	$-\Delta G^\circ_{300}$ (kcal mol^{-1})	A†	B†
1	7.6*	35.4	−3.0	1.66	7.73
2	5.1	33.1	−4.8	1.12	7.24
3	3.2	26.3	−4.7	0.71	5.75
4	3.1	27.0	−5.0	0.67	5.90
5	~3.2	~28.4	~−5.3	~0.7	~6.2

* From ref. 8.

† Constants for the equation $\log K_n = (A/T)\times10^3 - B$.

The $\Delta H_n{}^\circ$ values reported in Table 1 show that the $H_2CN^+\cdot nN_2$ cluster ions are very weakly bound and hence, collisional dissociation is expected to occur in the mass analysis system owing to collisions with 'background' gas. However, in a recent study on the $NO^+\cdot nN_2$ and $O_2{}^+\cdot nN_2$ formation, no evidence was found for this effect with respect to the equilibrium constant measurements.

Because N_2 is a weak base and has no dipole moment, the formation of large N_2 clusters is not expected. No results on the clustering of N_2 to H_2CN^+ leading to $H_2CN^+\cdot nN_2$ are available. The existence of these weakly bound cluster ions suggests that chemical bonding are also probably involved in their interactions. Hiraoka *et al.*[13] have shown that this is the case for $N_2H^+(N_2)_n$, $n=1$ to 6, that is the interactions are not

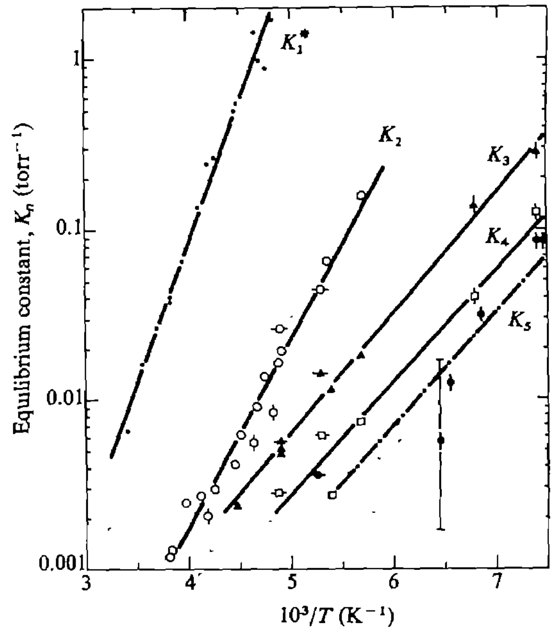

Fig. 2 Van't Hoff plots of equilibrium constants for reactions (1), (*from ref. 8). The dashed line for K_5 should be considered tentative, due to large measurement error (the estimated error for $T=155$ K, $n=5$, is shown). The estimated lower and upper limits for K_6, at $T=134$ K, are respectively 2.3×10^{-2} and 9.3×10^{-2} torr^{-1}. For clarity, K_6 is not drawn. Φ, ▲, ◫, ● 20 torr; ○, ▲, □, 40 torr; ⊖, ▲, ⊟, ●, 80 torr.

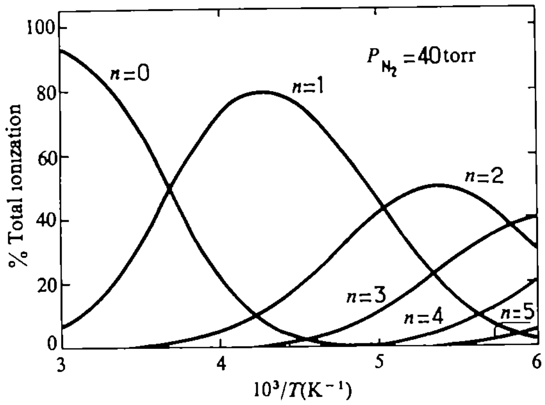

Fig. 3 Equilibrium distribution of ions $H_2CN^+ \cdot n\,N_2$ predicted by experimental data.

dominated by physical bonding (polarizabilities). There are some common features between the present results and those obtained by Hiraoka; for example (1) spacing between the lines of the van't Hoff plots shows a larger gap between the (0, 1) and (1, 2) equilibria; (2) the $\Delta H^{\circ}_{n-1,n}$ values are found to be almost constant between (2, 3,) and (4, 5); (3) these values are found to be nearly the same between (2, 3) and (4, 5) equilibria. The first feature indicates that $H_2CN^+ \cdot H_2$ and $N_2H^+ \cdot N_2$ are much more stable (towards loss of N_2) than the higher n complexes; the second feature shows that the stability decreases in that range are almost entirely due to entropy decreases. Finally, in both cases, $\Delta H^{\circ}_{n-1,n}$ decreases as n increases ending ultimately to the heat of vaporization of liquid N_2 (~1.3 kcal mol^{-1}).

The present results are consistent with the statement made by Capone *et al.*[4] on the possibility of $H_2CN^+ \cdot N_2$ association by three-body ion clustering reactions in the atmosphere of Titan. Furthermore they show that higher order associations are not excluded in this atmosphere.

Nevertheless, the high-altitude detection by Hartle *et al.*[11] of an ion of mass of the order of 28 AMU could also be interpreted as $C_2H_5^+$ (29 AMU) or N_2^+ (28 AMU). These ions are effectively observed in our mass spectra, as well as $C_2H_5^+ \cdot (N_2)_{1-5}$, $CH_5^+ \cdot (N_2)_{1,2}$ and $CH_3^+ \cdot (N_2)_{1,2}$. The lower limits for enthalpy and entropy changes for the three-body association reactions leading to $C_2D_5^+ \cdot (N_2)_{1,2}$ formation have been determined[14] recently in N_2–CD_4 mixtures. Moreover, the presence of small amounts of C_2H_2 in the atmosphere of Titan must have a very important effect on the ionic populations as we observe[15] in this experiment: ions such as $[(C_2H_2)_n(N_2)_m]^+$ and $X^+(C_2H_2)$ (where $X = C_3H_3, C_3H_4, C_3H_5, \ldots$) have been detected with very small admixture of C_2H_2 in N_2 and N_2–CH_4 mixtures respectively.

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Enhanced soft X-ray detection efficiencies for imaging microchannel plate detectors

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Position-sensitive detectors using microchannel plate (MCP) electron multipliers have been widely used in X-ray astronomy[1–3]. Although MCPs facilitate X-ray imaging with high spatial resolution, as on the Einstein[1] or EXOSAT[3] satellites, their intrinsic soft X-ray detection efficiencies are low. These lie typically in the range 1–10% (ref. 4), much lower than the (near unity) values attainable with competing forms of gas proportional counter. To enhance sensitivity to X-ray energies below a few keV, a material of relatively high photoelectric yield may be deposited on the MCP front surface and channel walls. MgF_2 has commonly fulfilled this role for X-ray astronomy[1–3], resulting in enhanced efficiencies 1.1–1.6 times those of uncoated MCPs[1]. We report here high 0.18–1.5 keV X-ray detection efficiencies for MCPs bearing CsI deposition photocathodes. Efficiency enhancement factors, dependent on X-ray incident angle, of up to 15 times have been stably obtained. The importance of this work for the sensitivity of future X-ray astronomy experiments, such as the Röntgensatellit (ROSAT) Wide Field Camera, is outlined.

A microchannel plate, formed by the drawing and etching of a lead glass matrix, consists of many (~10^6) hollow channels of common diameter 10–50 μm electrically connected in parallel. When a large bias voltage is applied along their length these channels can be made to act as a regular array of miniature photomultipliers. The many applications of MCPs have been reviewed by Wiza[5]. In the MCP's role as focal plane detector for grazing incidence X-ray optics[1,3] its above-mentioned inefficiency, even with the small enhancements provided by MgF_2 coating, has been a major factor in determining overall X-ray telescope sensitivities.

The adoption of MgF_2 as a 'standard' MCP coating for X-ray astronomy has been based on its relative photoelectric stability in a normal laboratory atmosphere[6] and not on its 'optimum' photoelectric yield properties in any absolute sense. A detailed study of X-ray photocathodes was therefore undertaken to identify, without experimental precondition, truly optimum candidate coating materials[7]. A collation of 1–300 Å X-ray photoemission data from many sources within a simple, predictive theoretical framework is given in ref. 7. CsI is found to be potentially a much superior MCP coating material to MgF_2 provided its hygroscopic nature—and hence possible instability in normal atmospheric humidity—can be accommodated by careful detector preparation and handling techniques.

Measurements were made on two position-sensitive X-ray detectors incorporating Mullard microchannel plates. The first of these (detector A) used a pair of MCPs of channel diameter $D = 12.5$ μm in a 'chevron'[5] arrangement. The input MCP (on which the CsI was deposited) had channels with a length-to-diameter ratio $L/D = 120:1$ biased at an angle $\phi = 4°$ to the front surface normal. The rear plate of this chevron was characterized by $L/D = 80:1$, $\phi = 13°$. The second detector (detector B) employed two 25-μm channel diameter MCPs, both of $L/D = 80:1$, with bias angles $\phi = 0°$ (input MCP) and $\phi = 13°$ (rear MCP).

The final stage in MCP manufacture is a reduction of the lead oxide glass content in a hydrogen atmosphere. New MCPs therefore copiously outgas water vapour and must be subjected to a prolonged vacuum bake before receiving a hygroscopic

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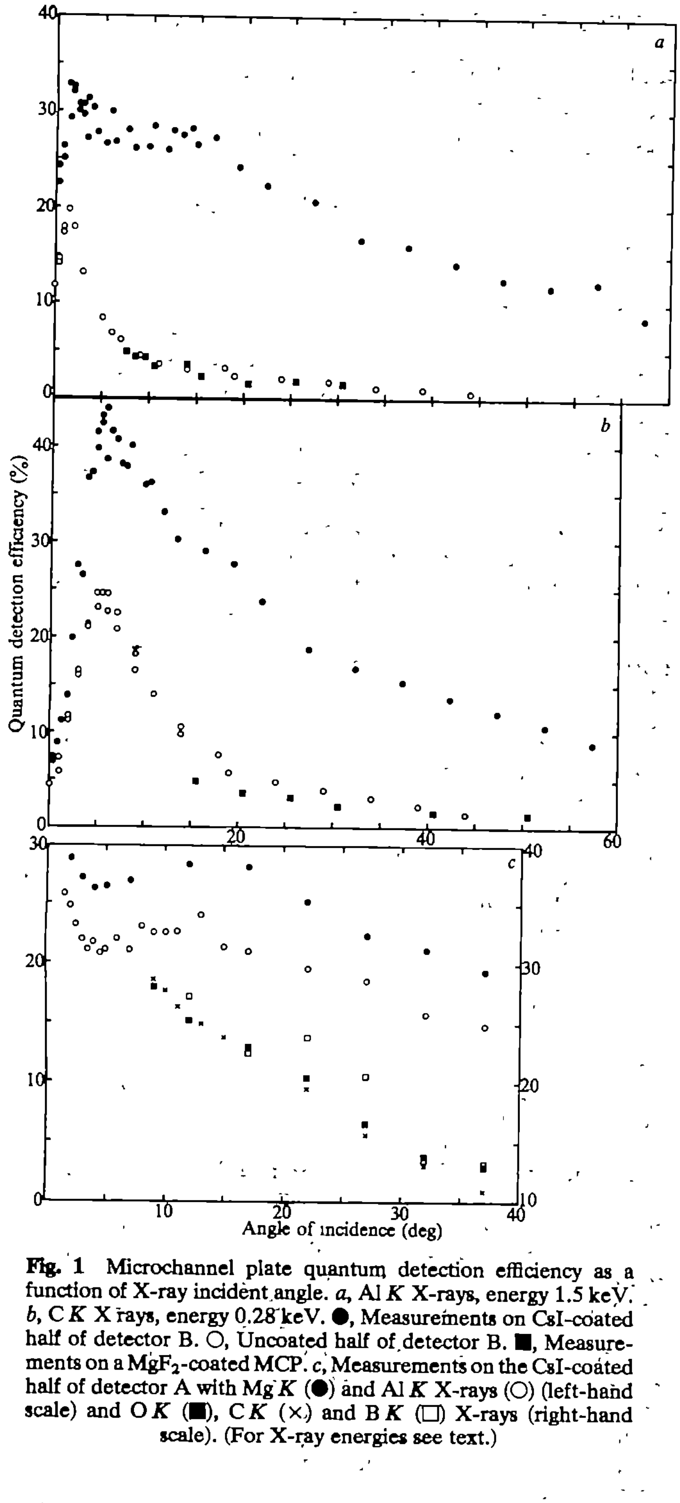

Fig. 1 Microchannel plate quantum detection efficiency as a function of X-ray incident angle. *a*, Al *K* X-rays, energy 1.5 keV. *b*, C *K* X rays, energy 0.28 keV. ●, Measurements on CsI-coated half of detector B. ○, Uncoated half of detector B. ■, Measurements on a MgF_2-coated MCP. *c*, Measurements on the CsI-coated half of detector A with Mg *K* (●) and Al *K* X-rays (○) (left-hand scale) and O *K* (■), C *K* (×) and B *K* (□) X-rays (right-hand scale). (For X-ray energies see text.)

coating. All our MCPs were vacuum-baked for 48 h at a temperature of 275 °C. The partial pressure of evolved H_2O was thus reduced by a factor of $\sim 10^3$, giving a final value of $\sim 10^{-10}$ torr (at room temperature) comparable with the partial pressures of secondary products—CO, N_2 and CO_2—revealed by mass spectral analysis.

CsI coatings were vacuum-evaporated onto one-half of each input MCP to a thickness of 6,000 Å on the MCP front surface. A nominal coating angle (between the molybdnum evaporation vessel and the normal to the centre of the MCP) of 15° was used for both detectors. The MCPs, preheated to 100 °C before CsI deposition, were rotated during the evaporation to ensure coating uniformity on the channel walls. The coating angle–front surface thickness combination used here produced a coating extending $\sim 3.7\,D$ down the channels with a characteristic thickness of 400 Å, about twice the escape length for X-ray-induced secondary electrons in CsI (ref. 7). The coated MCPs were then assembled into their respective detector bodies. All manipulations and transfers of the MCPs after bake-out took place in a local atmosphere of dry N_2 with the coated plates protected additionally by removable detector caps whenever possible. Thus, on entry into the vacuum chamber used for efficiency measurements, the CsI coatings had been exposed to air for only a few tens of seconds.

A comprehensive study of detector gain and noise count was made for each detector at operating pressures between 1 and 2×10^{-5} torr. Satisfactory peak gains and narrow pulse height distributions were obtained for both detectors. A peak gain $G = 3\times10^7$ and FWHM $\Delta G/G = 45\%$ were easily achievable with detector A. Noise count rates of the order of 0.5 $cm^{-2}\,s^{-1}$ (3–5 s^{-1} over total detector areas) were consistently obtained.

X-ray quantum detection efficiencies were measured for a range of X-ray energies and incident angles (the angle between the X-ray beam and the input MCP channel axis). The experimental technique was essentially that of Bjorkholm *et al.*[8]. The intensity of the incident X-ray beam, produced by a filtered, collimated fluorescence source, was measured using a pill-box type Ar–CH_4 (90:10 mixture) gas proportional counter[6] of known window absorption. Figure 1*a* displays detection efficiency versus incident angle for both CsI-coated and bare glass halves of detector B illuminated by Al *K* X rays of energy 1.5 keV. Use of a CsI deposition photocathode has dramatically increased the MCP efficiency at all angles. The prompt nature of the photoemission process (on time scales of 10^{-14} s) and the long dead time of an individual microchannel ($\sim 10^{-2}$ s)[5] dictate that the absorption of an X ray can give rise to at most one output pulse: the enhancement shown in Fig. 1*a* is not due to multiple pulses generated by a single photon. Figure 1*a* also shows measurements made in this laboratory on an MgF_2-coated MCP (M. Lewis, personal communication). The coating angles and thicknesses of the CsI and MgF_2 photocathodes were identical, although the pre-coating histories and quantum efficiencies of their MCP substrates differed. Figure 1*b* is the analogous figure for C *K* X rays, of energy 0.28 keV. All the efficiency–angle curves can be simply interpreted in terms[4] of (1) the increase in photoelectric yield with decreasing grazing angle to the channel walls until (2) the onset of X-ray reflection, (3) the fraction of X rays incident on the coated channel walls, (4) the geometric transparency of the MCP, (5) the divergence of the test beam (±0.5° FWHM).

Figure 1*c* shows efficiencies measured on the CsI-coated half of detector A for Al *K*, Mg *K* (1.25 keV), O *K* (0.525 keV), C *K* and B *K* (0.18 keV) X rays. Some spectral impurity of the O *K* line, arising from carbon contamination of the source anode, was possible with the present source–filter arrangement. Small-angle efficiencies have been omitted for clarity. Measured peak efficiencies in the order of decreasing X-ray energy were: 34.1, 35.6, 41.9, 48.1 and 38.3%. Detector A and B efficiencies are comparable, given the small differences between detectors due to bias voltage and channel geometry.

No measurements of the uniformity of the detection efficiency across the MCP surface were possible within the present vacuum system. A careful study of the photocathode deposition geometry has, however, been made to ensure that variations in the CsI coating profile were minimized within and between channels.

Repeat measurements on detector A were done to establish the ability of the CsI coating to withstand the vacuum breaking inevitable in the development of a flight detector for X-ray astronomy. No significant changes in gain, noise count or efficiency were noted during 11 cycles between vacuum and a dry N_2 environment, involving an accumulated input to the CsI of 5×10^5 photons mm^{-2} over a period of 18 days under continuously pumped vacuum. Detector B, by contrast, was subjected to several deliberate exposures to laboratory air. Relative humidity on exposure varied between 42 and 53% with ambient temperatures of 22–25 °C. No degradation in CsI efficiency was observed even after an accumulated exposure time of 8 h. Work continues to establish the long term stability of the response of CsI-coated MCPs under various methods of storage. We note here that planar CsI XUV photocathodes have been found to be stable after 9 months of storage in dry N_2 (ref. 9).

These preliminary measurements indicate that CsI deposition photocathodes can provide a simple, stable and reproducible means of producing high efficiency microchannel plate X-ray detectors both for X-ray astronomy and other imaging applications[5]. We stress that the efficiencies reported here do not represent the limits attainable using a deposition photocathode technique. A thicker CsI layer, deposited at smaller coating angle, should appreciably increase peak efficiencies. Further, in the work discussed above, there is no contribution from the inter-channel area (37% of the MCP front surface) to the detection efficiency because of its large negative potential (~ -3.5 kV). As the input MCP is preceded by an earthed carbon-coated plastic film, photoelectrons released from the front surface are directed away from the channel entrances[4]. The use of a high transparency grid to establish an appropriate electron collecting field at the MCP input may be expected to increase detection efficiencies at all angles of incidence.

Calculations based on the work described in refs 4 and 7 and supported by the present data, indicate that an MCP efficiency of 45% should be attainable for an X-ray energy of 0.1 keV and an incident angle of 30°. Such a combination is directly relevant to the Wide Field Camera (WFC), an XUV telescope now being developed by a consortium of UK research groups for flight on the German national ROSAT[10] in 1987. The WFC is intended to carry out the first XUV (0.04–0.2 keV) sky survey. Use of a CsI-coated MCP is expected to increase the WFC sensitivity by a factor ~4 times relative to the MgF_2-coated detector of the design study (R. Willingale, personal communication).

Calculations also indicate an efficiency of at least 20% for 6-keV X rays incident at 4° to the channel axes, about six times the corresponding efficiency of an uncoated MCP. This energy-angle combination is of interest for focal plane instrumentation for AXAF[11], NASA's principal mission in X-ray astronomy planned for the next decade.

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Seasonal effects of temperature and salinity on the partial pressure of CO_2 in seawater

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The exchange of CO_2 between the atmosphere and the sea is of major importance to our understanding of the climatic consequences of anthropogenic CO_2. Because the solubility of CO_2 and the dissociation of carbonic acid in seawater are modulated by temperature and salinity, the detection of long-term changes in the oceanic carbonate system, first requires that the comparatively large short-term variations in the partial pressure of CO_2 should be characterized. We present here the first direct observations of seasonal variations in the partial pressure of CO_2 in the subtropical gyres of the North and South Pacific Ocean. These variations compare well with the variations predicted from observed changes in temperature and salinity using empirical equations which do not require the determination of alkalinity or total inorganic carbon, and the agreement is improved by the inclusion of air–sea exchange in the model calculations. Thus we predict that specific alkalinity within the two gyres remains extremely constant, which is confirmed by carbonate equilibrium calculations based on our measurements of total inorganic carbon.

The measurements were carried out as part of the NORPAX Equatorial Experiment during a series of month-long oceanographic expedition legs extending between Papeete, Tahiti and Honolulu, Hawaii. Continuous shipboard measurements of CO_2 partial pressures in surface waters were carried out between August 1979 and June 1980 aboard RV *Wecoma* on the northbound expedition Legs 7, 9, 13 and 15. The analyses were made using automated dual-catalyst flame ionization gas chromatography[1] to determine the mole fraction of CO_2 in a dried aliquot of a gas phase which was equilibrated continuously with seawater pumped from a depth of ~3 m. The results were corrected for slight warming in the pumping and equilibration system using the empirical temperature dependence given below (equation (1)). Measurements of atmospheric CO_2 mole fractions were also made by the shipboard chromatographic system. Standard deviation of replicate chromatographic CO_2 measurements is ~0.04%, and the estimated systematic uncertainty of these measurements, including uncertainties associated

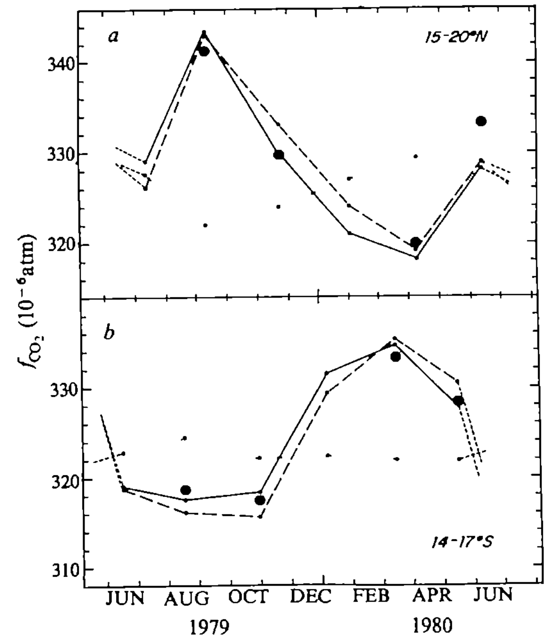

Fig. 1 Seasonal variations in the equilibrium fugacity of CO_2 in surface ocean water of the tropical limbs of the North Pacific (*a*) and the South Pacific (*b*) subtropical gyres. The regional mean measured seawater fugacities are plotted as solid circles, and the fugacities of atmospheric CO_2 at the water-saturated air–sea interface are connected by dotted lines. Surface ocean water fugacities predicted from temperature and salinity without air–sea exchange are shown as dashed lines, and with air–sea exchange are shown as solid lines (see text).

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Table 1 Mean properties of surface ocean water and the atmosphere during the NORPAX equatorial experiment

Leg no.	Date	Seawater f_{CO_2} (μatm)	t (°C)	S (‰)	Normalized A_T* (μequiv. kg^{-1})	Atmosphere f_{CO_2}† (μatm)
North Pacific subtropical gyre (15–20° N, 158° W)						
5	14 July 1979	—	25.99	34.477	—	327.6
7	10–11 September 1979	341.3	27.11	34.682	2,319.8	322.0
9	23–24 November 1979	329.8	26.31	34.685	2,317.9	323.9
11	2–3 February 1980	—	25.51	34.737	—	327.0
13	9–10 April 1980	319.9	25.13	34.722	2,322.0	329.5
15	13–14 June 1980	333.2	25.98	34.672	2,317.7	328.8
South Pacific subtropical gyre (14–17° S, 150° W)						
5	18 June 1979	—	27.58	35.798	—	322.9
7	19 August 1979	318.6	27.03	36.080	2,321.6	324.4
9	1 November 1979	317.6	26.97	36.099	2,319.7	322.2
11	8 January 1980	—	28.54	35.737	—	322.4
13	18 March 1980	333.3	29.09	35.696	2,319.9	321.9
15	18 May 1980	328.3	28.94	35.481	2,317.2	321.9

* Mean values of A_T calculated from measured ΣCO_2 and f_{CO_2} (see text) and normalized to $S = 35$‰.
† Fugacities at the water-saturated air–sea interface.

with the calibration of compressed air standards by nondispersive IR analysis and manometric techniques[2], is ~0.05%. An additional estimated uncertainty of 0.5% is associated with possible systematic effects in the seawater equilibration process.

Measurements of atmospheric CO_2 were also made by shorebased nondispersive IR analysis[2] using flask samples collected in triplicate on all the NORPAX expedition legs. The standard deviation of replicate flask measurements is ~0.06%, with an additional systematic uncertainty of ~0.04%.

Seawater samples for shorebased determination of total inorganic carbon (ΣCO_2) were collected at hydrographic stations on northbound odd-numbered expedition legs using Niskin bottles at a depth of 10 m. The samples were collected in duplicate and were preserved with $HgCl_2$. The ΣCO_2 measurements were made by acidifying a weighed sample and trapping the evolved CO_2 with liquid nitrogen in a vacuum system. The CO_2 was separated from H_2O with a dry ice trap and was then determined manometrically with the same constant volume manometer used to determine the mole fraction of CO_2 in air[2]. The standard deviation of replicate samples is 0.7 μM kg^{-1}, and the accuracy, as judged from artificial samples, is ~1.0 μM kg^{-1}.

The measured concentrations of CO_2 in the equilibrated gas and in the atmosphere were converted to fugacities for the purpose of evaluating real-gas equilibrium relationships, using the virial equation of state[3] and assuming a water-saturated interface at the measured barometric pressure. The solubility of CO_2 in seawater as a function of fugacity was taken from the equations in ref. 3. Calculations of carbonate system equilibria were carried out using the dissociation constants in seawater of carbonic acid, boric acid and water, as measured on the hydrogen ion concentration scale by Hansson[4] and fitted to the equations of Millero[5]. These values were chosen in preference to the measurements on the NBS buffer scale[6–8], principally because the Hansson values for boric acid are in better agreement with the work of Millero[9] and with the recent measurements of A. Dickson (personal communication) than are the Lyman values[7]. Note, however, that the results presented below are not strongly dependent on the choice of dissociation constants[5].

The temperature dependence of the fugacity of CO_2 (f_{CO_2}) in a gas phase in thermodynamic equilibrium with seawater at constant total titration alkalinity (A_T) and constant ΣCO_2 was determined empirically from calculated equilibria. Values of f_{CO_2} were calculated over the ranges of 0–36 °C in temperature (t), 30–38‰ in salinity (S), 64–68 μequiv. kg^{-1} ‰$^{-1}$ in specific alkalinity (A_T/S), and 1.05–1.20 μequiv. μM^{-1} in the $A_T/\Sigma CO_2$ ratio. The resulting values of f_{CO_2} bracket the atmospheric range at all temperatures and salinities and include extrema from 80 to 2,000 μatmospheres (atm). For each combination of ΣCO_2, A_T and S, $\ln(f_{CO_2})$ was fitted by least squares to a power series in t. These expressions were then differentiated to obtain values of $\partial \ln(f_{CO_2})/\partial t$, which were again fitted to the equation

$$\frac{\partial \ln(f_{CO_2})}{\partial t} = 0.03107 - 2.785 \times 10^{-4}\, t - 1.839 \times 10^{-3} \ln(f_{CO_2}) \quad (1)$$

which represents the temperature dependence of the calculated fugacities with a standard deviation of 0.05% °C^{-1}. The precision of this fit greatly exceeds those of previous empirical representations[10].

The salinity dependence of f_{CO_2} at constant specific alkalinity and constant specific total inorganic carbon ($\Sigma CO_2/S$) was determined in an analogous fashion to the temperature dependence, using values of f_{CO_2} calculated over the same ranges of t, S, A_T, and $A_T/\Sigma CO_2$. For each combination of t, A_T/S and $\Sigma CO_2/S$, $\ln(f_{CO_2})$ was fitted to a power series in S, differentiated to obtain values of $\partial \ln(f_{CO_2})/\partial S$, and fitted to the equation

$$\frac{\partial \ln(f_{CO_2})}{\partial S} = 0.08620 - 1.272 \times 10^{-3}\, S \quad (2)$$

which represents the salinity dependence of the calculated fugacities with a standard deviation of 0.06% per ‰ salinity.

An important characteristic of equations (1) and (2) is their independence from A_T and ΣCO_2, which makes it possible to evaluate the effects of temperature and salinity variations on f_{CO_2} in nature and in experimental applications without the need to determine other parameters of the dissolved carbonate system. Both equations are easily integrated in modelling applications, without significant loss in accuracy compared with equilibrium calculations for waters with well-characterized carbonate systems.

The results of the NORPAX f_{CO_2} measurements in the North and South Pacific subtropical gyres are summarized in Table 1. The mean values listed are confined to the tropical limbs of the gyres north of 15° N and south of 14° S, where the continuous chromatographic measurements do not show the increase in f_{CO_2} due to upwelling in the equatorial region[11]. To facilitate comparisons between the chromatographic measurements and the hydrographic observations spaced at 1° latitude intervals[12], the tabulated values include only the chromatographic measurements at the individual station locations. In addition to the data for expedition Legs 7, 9, 13 and 15, on which the chromatographic measurements were made, Table 1 includes hydrographic and atmospheric CO_2 flask data for adjoining Legs 5 and 11.

The seasonal variations of surface water f_{CO_2} in both gyres are plotted in Fig. 1. The peak-to-peak amplitude of the variations is about 20 μatm, with both hemispheres showing summer maxima. The predicted variations in f_{CO_2}, calculated from the observed changes in temperature and salinity by integration of equations (1) and (2) with the fugacity boundary condition adjusted to minimize the r.m.s. deviation of the measured fugacities, are plotted for each hemisphere as dashed lines in Fig. 1. The agreement between the measurements and these calculations, which do not allow for air–sea exchange or for seasonal variations in the admixture of waters with other carbonate chemistry, is nonetheless striking, with r.m.s. deviations of 3.0 and 2.3 μatm in the northern and southern gyres respectively. Thus, the observed variations in both hemispheres are due mainly to seasonal variations in surface water temperature and salinity, rather than to removal or addition of CO_2 by physical or biological processes.

We have considered the effect on f_{CO_2} of biological activity, as reflected in the surface water nutrient concentrations[12]. Assuming carbonate chemistry equilibrium, the Redfield, Ketchum and Richards mean planktonic composition[13], and the complete assimilation of available nitrate, the maximum predicted f_{CO_2} variation is 0.3 μatm in the northern gyre and 0.6 μatm in the southern gyre. However, inasmuch as the measured surface water nitrate values in these regions remain at or below the 0.1 $\mu M\,kg^{-1}$ detection limit, we have neglected this small effect in our model calculations.

The solid lines in Fig. 1 show the effect of including air–sea exchange in the calculations. This was done by a simple numerical integration, in which the CO_2 flux was assumed to be proportional to the fugacity difference between the surface water and the measured atmosphere, the mean exchange rate was taken as $22\,M\,m^{-2}\,yr^{-1}$ (refs 14, 15), the mean depth of the mixed layer determined from the hydrographic data was 70 m, the mean values of t, S and atmospheric f_{CO_2} were interpolated linearly between legs, and the mean buffer factor $\partial\ln(f_{CO_2})/\partial\ln(\Sigma CO_2)$ calculated from the chemical constants discussed above was 8.35 in the northern gyre and 8.50 in the southern gyre. As in the case of the conservative calculation, the fugacity boundary condition in each hemisphere was adjusted to minimize the r.m.s. deviation of the measured seawater fugacities. The main effect of adding air–sea exchange to the calculations is a slight advance in the phase of the predicted variations, which further reduces the r.m.s. deviations to 2.6 and 1.1 μatm in the northern and southern gyres respectively.

This excellent agreement between the measured values of f_{CO_2} and the predicted variations for a model which is conservative in CO_2 except for air–sea exchange, requires that the salinity-normalized alkalinity in both gyres be constant to within about 2 $\mu equiv.\ kg^{-1}$. To test this hypothesis, we have calculated A_T from our ΣCO_2 and f_{CO_2} measurements using the chemical constants discussed above. The mean values of A_T normalized to $S = 35‰$ are listed in Table 1. The averages and standard deviations of the normalized values of A_T for the four expedition legs are $2{,}319.3 \pm 2.0\ \mu equiv.\ kg^{-1}$ in the northern gyre and $2{,}319.6 \pm 1.8\ \mu equiv.\ kg^{-1}$ in the southern gyre. The results thus confirm the remarkable seasonal constancy of specific alkalinity in the tropical limbs of the Pacific subtropical gyres, and demonstrate that seasonal variations in their CO_2 fugacities may be calculated directly from changes in temperature and salinity, with a small correction for air–sea exchange.

Note that the seasonally averaged surface water CO_2 fugacities in the tropical limbs of both gyres are significantly greater than the seasonally averaged atmospheric fugacities (Fig. 1), and there is therefore a net flux of CO_2 from the sea to the atmosphere of the order of $0.1\,M\,m^{-2}\,yr^{-1}$. While part of this flux may be due to upwelling across the thermocline, we expect the major source to be thermal pumping of atmospheric CO_2 which dissolves in the cooler high-latitude limbs of the gyres and is subsequently liberated as these surface waters are advected into the tropics and warmed. However, seasonal measurements of CO_2 in higher latitudes are needed to determine the relative importance of these mechanisms.

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A new type of decollement thrusting

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One of the fundamental rules of decollement tectonics is that decollement horizons form in mechanically weak layers[1,2]. Here we document two examples of decollement-style thrust faults that detach within thick platformal dolostones in preference to apparently weaker layers of shale, siltstone and limestone underlying the dolostones. The thrusts are the Keystone–Muddy Mountain–Glendale thrust system (the KMG thrust system), , and the underlying Contact–Red Spring–North Buffington–Mormon thrust system (the CRM thrust system), both of southern Nevada. They form part of the Sevier orogenic belt, and extend for roughly 250 km along strike, together showing at least 65 km tectonic overlap (Fig. 1). Although the thrust systems are closely related spatially, the higher KMG system is younger than the underlying CRM thrust system, and the two developed essentially independently[3–7]. Three points are important: first, that both the KMG and the CRM thrust systems are decollement style thrusts; second, that the decollement horizon is primarily restricted to a narrow stratigraphical interval within a bedded sequence of essentially homogeneous dolostones of the Middle Cambrian Bonanza King Formation; the third, that the thrust faults formed at a very shallow crustal level (<5 km).

Detailed field studies in the past 15 yr have yielded a wealth of structural and stratigraphical data for the thrust systems in question[5–12]. To demonstrate a decollement geometry for any foreland thrust, it is sufficient to show that the thrust fault carries the same structural or stratigraphical unit in the hanging wall for a large distance across strike (in the transport direction). If this criterion is met, the thrust fault must have decollement geometry, regardless of later deformations. The two thrust systems are discussed separately—first the CRM thrust system, then the KMG thrust system.

Both the KMG thrust system and the CRM thrust system carry the Banded Mountain Member of the Bonanza King

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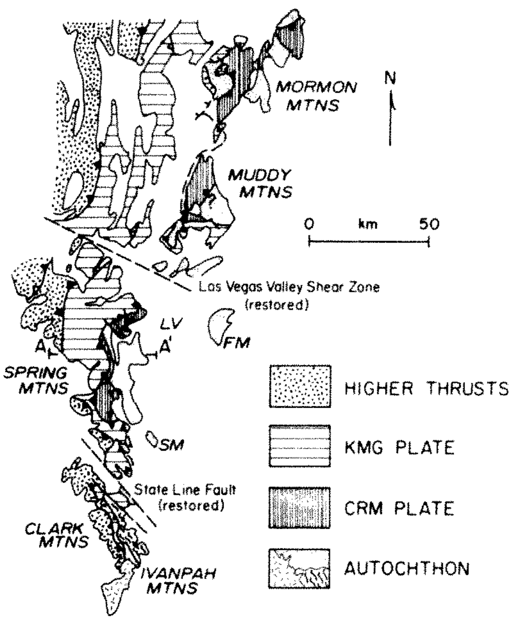

Fig. 1 Simplified tectonic map of the Keystone–Muddy Mountain–Glendale and Contact–Red Spring–North Buffington–Mormon thrust systems. Right-lateral movement on the Tertiary Las Vegas Valley shear zone and Pahrump-State Line fault zone has been removed. Thrust transport direction is roughly east-northeast[9]. LV, Las Vegas; FM, Frenchman Mountain; SM, Sheep Mountain.

Formation in the hanging wall for great distances along and across strike. With a few exceptions, the thrust faults virtually everywhere occur within 75 m of a distinctive marker horizon at the base of the Banded Mountain Member[10,11,13]. The Middle and Upper Cambrian Bonanza King Formation (for original definition, see ref. 14) is the basal unit of the thick Palaeozoic miogeoclinal platform carbonate sequence of the southern Great Basin. Two members are commonly identified: the upper Banded Mountain Member and the lower Papoose Lake Member[9,13,15]. The Banded Mountain Member generally consists of well-bedded dolostone with rare limestone in the region of Fig. 1; the Papoose Lake Member generally consists of mottled dolostone and limestone[13]. The Banded Mountain Member averages ~500 m in thickness where a complete section is exposed; the Papoose Lake Member varies between 175 and 450 m thick, where exposed (Fig. 2).

The Banded Mountain Member forms the hanging wall of the Mormon thrust fault for a distance of at least 40 km in the transport direction[7]. The Mormon thrust fault is probably correlative with the North Buffington thrust in the central Muddy Mountains, and the Red Spring and Contact thrusts in the central Spring Mountains[6,7]. All of these correlative thrusts carry the Banded Mountain Member of the Bonanza King Formation in the hanging wall[6,12]. The CRM thrust system carries the same unit in the hanging wall for at least 40 km across strike and at least 150 km along strike; it therefore has a decollement geometry.

Thrust faults in the KMG thrust system carry the Banded Mountain Member of the Bonanza King Formation in the hanging wall for 15 km across strike in the northern Clark Mountains[5,8], for 8–10 km across strike in the central Spring Mountains[10,16], and for at least 25 km across strike in the Muddy Mountains[6]. The KMG thrust system carries the Banded Mountain Member in the hanging wall for nearly 200 km along strike, from the southern Mormon Mountains in the north[7], through the Muddy Mountains[6] and Spring Mountains[5,9] to the Clark and Ivanpah Mountains of southeastern California[8]. Therefore, the KMG thrust system also has decollement geometry, by the criterion above.

The phrase 'in the hanging wall' is used to denote that part of the upper plate immediately adjacent to the thrust fault itself: 'hanging wall' means the bottom surface of the upper plate of the thrust, and not volume of the upper plate.

Because the Banded Mountain Member forms the hanging wall for virtually all of the exposures of both thrust systems, it is clear that this unit contains the decollement horizon for the exposed portions of the thrusts. To demonstrate that a decollement horizon formed within the Bonanza King Formation in preference to the underlying Middle and Lower Cambrian limestones, shales and siltstones, it must be shown that the Papoose Lake Member was present beneath the Banded Mountain Member in the allochthon before formation of the thrust faults.

Complete sections of Cambrian strata are exposed in the autochthon in the Mormon Mountains, at Frenchman Mountain, at Sheep Mountain and in the Ivanpah Mountains (see Fig. 1). All three of these sections contain a thick Papoose Lake Member[7,9,17,18]. The Papoose Lake Member is also present in the higher allochthons west of the KMG thrust system[8,9,19,20]. The Papoose Lake Member and underlying strata are regionally extensive, present everywhere the entire Cambrian section is exposed both east and west of the two thrust systems discussed here. It follows that these beds were originally present beneath the Banded Mountain Member of both the KMG and CRM allochthons, and were left behind beneath the decollement during thrust faulting. This is supported by the occurrence of slivers of the Papoose Lake Member preserved locally above the thrusts[12,18].

It is possible that the decollement in the Banded Mountain Member is not a basal decollement, but rather the roof fault for a duplex structure at depth. There is no structural evidence for such a duplex: if one exists it is nowhere exposed, and an internally consistent set of balanced cross-sections can be drawn without postulating such a structure. In addition, the CRM thrust system, overlying KMG thrust system, and still higher Green Monster–Lee Canyon thrust system all carry the basal beds of the Banded Mountain Member in the hanging wall in virtually every exposure; the Papoose Lake Member is present only as slivers, and lower units are not present at all. The simplest explanation is to postulate a master decollement near the base of the Banded Mountain Member.

We infer that the CRM and the KMG thrust systems formed at shallow levels on the basis of the general absence of ductile deformation within the thrust sheets and the presence of fore-thrust debris and stream-channel conglomerates in the footwall of both thrust systems. The absence of pervasive ductile defor-

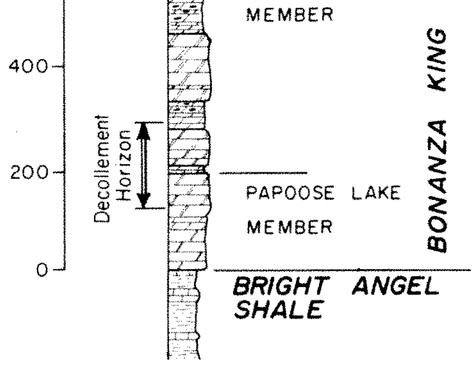

Fig. 2 Generalized stratigraphical column of the Bonanza–King Formation in the area of Fig. 1. Decollement horizon of the Keystone–Muddy Mountain–Glendale and Contact–Red Spring–North Buffington–Mormon thrust systems lies at base of the Banded Mountain Member.

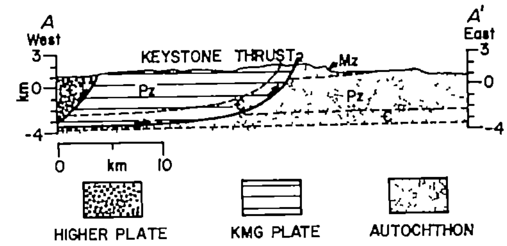

Fig. 3 Cross-section of the Keystone thrust system in the central Spring Mountains (A–A' in Fig. 1), showing decollement nature of the fault and simple ramp geometry. No vertical exaggeration.

mation merely implies that the thrusts did not form in metamorphic conditions. The presence of forethrust debris beneath the thrust faults implies that both thrust systems were 'erosional thrusts'; thrust systems whose fault plane lay at or near the surface of the Earth during thrusting, and whose upper surface was a topographic surface during thrust advance[21–23,30]. A typical cross-section through the central Spring Mountains (Fig. 3) shows that the Keystone thrust fault forms a single ramp from the decollement horizon in the Banded Mountain Member of the lower plate to the ancient erosional surface. The autochthonous section is roughly 4.5 km thick at the Keystone ramp; therefore the thrust fault at the base of the ramp must have been no more than 5–5.5 km below the surface at the time of formation, allowing for post-Keystone erosion. A similar argument holds for the CRM thrust system.

One important aspect of the Banded Mountain Member decollement surface is its very large area. Palinspastic restoration of the displacements on the two thrust systems provides an estimate of the initial size of the decollement surface. The length along strike is at least 200 km, from exposures of the KMG thrust system. Adding values of tectonic overlap for the KMG and CRM thrust systems will give a minimum value for the across strike extent of the decollement surface. Minimum overlap on the CRM system is 40 km (ref. 7); minimum overlap on the KMG system is 25 km (ref. 6). The decollement formed in the Banded Mountain Member must then be at least 65 km wide; the total area of the decollement must be at least 13,000 km^2.

The principal mechanism of deformation near the thrust faults appears to be cataclasis[24]. Moderate to intense brecciation is common in the upper plate up to several tens of metres away from the fault[7,8,10–12,18,25]. Evidence for extensive ductile deformation within the thrust sheets is present only in the Clark Mountains at the southernmost end of the Keystone thrust, where late syn-tectonic plutonism provides a source for heat and fluids[8,18]. Evidence for ductile deformation near the thrust fault disappears a short distance away from the plutons.

Of paramount importance in the problem of formation of this decollement surface is that there are no lithological reasons for postulating any special mechanical properties for the rocks of the Banded Mountain Member in the decollement zone: there are no shaley layers, no gypsiferous layers, nothing but monotonous fine-grained laminated dolostone locally including secondary, recrystallized coarse-grained dolomite (Fig. 2). This has been established by detailed stratigraphical and petrological studies in both allochthonous and autochthonous sections[5,16]. The formation of the decollement horizon appears to be controlled by factors other than lithological contrasts within the decollement zone.

The key mechanical problem is not the transport of these thrust systems, although that problem is complex, and despite recent purely theoretical models[23,26] is still poorly understood. The main mechanical problem is: why did the decollement form within the dolostones, ignoring shale only a few hundred metres below? Our present view is that when subjected to horizontal compression the highly competent dolostones acted as a stress guide, concentrating horizontal stress within the dolostones unit with respect to the weaker materials below[27]. Preliminary modelling studies by one of us (J.H.W.) indicate that this variation in horizontal stress may have a key role in the localization of the decollement horizon.

The observation that the thrusts formed at shallow crustal levels is very important in light of the low strength of shale, siltstone and limestone in brittle conditions[28]. Our finding that the decollement formed within the strong dolostones in preference to the apparently weaker underlying layers demonstrates that a low-strength layer at the decollement horizon is neither a necessary nor a sufficient condition for the formation of a decollement thrust in the orogenic foreland. Existing theories of foreland thrusting appear inadequate to explain the formation of these decollement zones[2,29]. We suggest that the Keystone–Muddy Mountain–Glendale thrust system and the Contact–Red Spring–North Buffington Mormon thrust system represent type examples of a class of 'strong' decollement' foreland thrusts.

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The La–Ce geochronometer: a new dating method

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The isotope ^{138}La(0.089% of natural lanthanum) decays by β^- and electron capture to ^{138}Ce and ^{138}Ba with a long half-life. We present here the first age determination using La–Ce dating. An internal La–Ce isochron of a gabbro from the upper zone of the Bushveld, South Africa, yields an age of 2,390 ± 480 (2σ) Myr based on the partial decay constant 2.58×10^{-12} yr^{-1}. This age agrees with the Sm–Nd isochron age (2,050 ± 90(2σ) Myr) which was obtained from an aliquot of the same sample solution: within the uncertainties of ^{138}La half life and isotopic measurement.

Radioactivity of ^{138}La has been predicted, because of Mattauch's rule concerning the stability of isobaric atomic nuclei

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Table 1 Summary of partial and total half life data and radioactivity determination of ^{138}La

Partial half life (10^{11} yr)		EC decay / β decay	Total half life		
β decay	EC decay		(10^{11} yr)	Method	Refs
			2.0	γ counting with NaI	2
12	0.7	0.06	0.7	β counting with Al absorbent	3
2.4±0.2	2.1±0.1	0.88±0.08	1.0±0.1	γ counting with NaI	4
3.5±0.3	1.64±0.06	0.47±0.04	1.12±0.10	γ counting with NaI	5
4.1±0.7				β counting with proportional tube	5
2.83±0.04	1.66±0.02	0.59±0.01	1.04±0.014	γ counting with NaI	6
4.7±1.5	2.34±0.37	0.50±0.18	1.56±0.3	γ counting with Ge(Li)	7
3.95±0.63	1.87±0.21	0.47±0.09	1.27±0.18	γ counting with Ge(Li)	8
3.68±0.14	1.99±0.03	0.54±0.02	1.29±0.05	γ counting with Ge(Li)	9
		0.51±0.04	1.28±0.12	γ counting with Ge(Li)	10
3.02±0.04	1.56±0.05	0.51±0.01	1.03±0.02	γ counting with Ge(Li) and pure Ge	11
2.95±0.15*	1.74±0.13		1.05±0.05		
2.69±0.24†	1.51±0.10		0.94±0.06		

* Weighted mean of the data from refs 4–11.

† Weighted mean of the data from refs 4–10 which were multiplied by 1/1.17 (see text) and raw data of ref. 11.

($^{138}_{56}$Ba–$^{138}_{57}$La–$^{138}_{58}$Ce) with contiguous atomic numbers and the half-life has been measured[1–11,25]. ^{138}La decays by β^- emission to an excited level of ^{138}Ce which emits 789 keV γ ray and by electron capture to an excited level of ^{138}Ba which emits a 1,436 keV γ ray. The published values for the half lives of these two decay processes and weighted average values are summarized in Table 1. These partial half lives as well as the total half life scatter rather widely, while the branching ratios of the two decays are in reasonable agreement. Reported values of half lives with no information on volatile impurities were uniformly reduced here by a factor of 0.855 (=1/1.17) assuming that the oxide measured contained 17% volatiles due to absorption of H_2O and CO_2. The weighted average values are also listed in Table 1.

An upper zone gabbro of the Bushveld complex was sampled at Magnet Height, is composed of about 50% pyroxenes, 40% plagioclase and minor amounts of biotite, hornblende and magnetite. The gabbro was crushed and pyroxenes and plagioclase were separated from both 60–80 and 80–140 mesh fractions by isodynamic separator.

About 0.5–1 g for each sample (two pyroxenes, two plagioclases and a bulk rock) was decomposed by HF and $HClO_4$. The samples were then dissolved in HCl and separated into two aliquots, and composite REE spike was added to one of them. Ce and Nd were chemically separated for the measurement of isotopic abundance ratios involved three ion-exchange columns, and, Nd was finally eluted by 0.25 M α-hydroxyisobutyric acid (α-HIBA), and Ce by 0.3 M α-HIBA, respectively. Overall blanks were 0.4 ng for Nd and 3 ng for Ce. The moderately high blanks were due to the large amounts of reagents used for large samples. However, even for the most Ce-poor pyroxene, the sample/blank ratio was more than 800 for both elements, and hence no correction for blanks on isotopic ratio was made. Blank values for abundance determination by isotope dilution were 10 times smaller than those for isotopic composition. Tiny CeO^+ peaks with natural isotopic ratios were detected from the outgassed filament system, and the beam intensity of mass 158 ($^{142}Ce^{16}O$) was ~5×10^{-16} A in the same operating condition as normal running with a sample. This background intensity was less than 1/10,000 of the sample beam intensity, and the effect was included in the blank value mentioned above.

Cerium isotopes were measured as CeO^+ on Re triple filaments. The peaks of masses 152 ($^{136}Ce^{16}O$), 154 ($^{138}Ce^{16}O$) and 158 ($^{142}Ce^{16}O$) were measured. The biggest peak of mass 156 ($^{140}Ce^{16}O$) was ignored in order to get good measurements for the tiny peaks of masses 152 and 154. Masses 153, 155, 157 and 162 were usually monitored. Although the small 155 ($^{139}La^{16}O$) and 157 ($^{141}Pr^{16}O$) peaks were observed in most cases, the 154/155 and 158/157 intensity ratios were greater than 50 and 1,000, respectively. Very small BaO^+ and BaF^+ peaks were detected only at an early low temperature stage and soon disappeared with growth of the CeO^+ beam. NdO^+ was observed in a few cases and corrected where necessary. The beam intensity of mass 158 ($^{142}Ce^{16}O$) was 0.5 to 1.0×10^{-11} A. Generally 100 scans of the peaks were accumulated for each run. Each run took 3–4 h and 2–4 runs were measured for every sample. $^{136}Ce/^{142}Ce = 0.01720$ was used for normalization. The observed fractionation factor between ^{136}Ce and ^{142}Ce was used to estimate the $^{140}Ce/^{142}Ce$ ratio, followed by an evaluation of $^{140}Ce^{18}O^+$ effect on $^{142}Ce^{16}O^+$. $^{140}Ce/^{142}Ce$ was taken to be 7.992 at $^{136}Ce/^{142}Ce = 0.01720$, that is, with no mutual fractionation. However, the mass fractionation correction for $^{140}Ce^{18}O$ was very small, and almost constant. The Johnson Matthey Ce reagent, JMC 304, was measured with a rather wide range of ion current ($0.3\sim2\times10^{-11}$ A) and isotope fractionation [($^{136}Ce/^{142}Ce$) fractionation = $1.0001\sim0.996$]. No systematic variation was found. The mean $^{138}Ce/^{142}Ce$ value for 15 runs on the reagent was 0.0228559±0.0000011 (2σ).

Neodymium was measured as NdO^+ by a Re single filament. Small PrO^+ and SmO^+ peaks were sometimes observed and $^{147}Sm^{16}O$ was monitored for Sm correction if needed. All the measured isotopic ratios were normalized against $^{146}Nd/^{144}Nd = 0.7219$. La Jolla Nd Standard reagent (provided

Table 2 La–Ce and Sm–Nd isotopic data for Bushveld upper zone gabbro

Sample	La (p.p.m.)	Ce (p.p.m.)	$^{138}La/^{142}Ce$*	$^{138}Ce/^{142}Ce$†	Sm (p.p.m.)	Nd (p.p.m.)	$^{147}Sm/^{144}Nd$*	$^{143}Nd/^{144}Nd$†
60–80 mesh fraction								
Pyroxene	1.010	3.91	0.00209	0.022848±4	1.255	3.81	0.1993	0.51230±5
Plagioclase	2.39	3.74	0.00518	0.022868±9	0.1391	1.123	0.0749	0.51066±7
80–140 mesh fraction								
Pyroxene	0.640	3.01	0.00172	0.022843±7	0.989	2.97	0.2014	0.51235±5
Plagioclase	2.35	3.71	0.00514	0.022864±8	0.1350	1.082	0.0755	0.51062±7
Bulk	1.743	4.23	0.00334	0.022856±5	0.689	2.53	0.1647	0.51189±4

* $^{138}La/^{142}Ce = (La/Ce)_{weight}\times0.00811$. The factor was based on ^{138}La abundance (0.00089), ^{140}Ce abundance (0.1107) and atomic weights 138.91 and 140.12 for La and Ce, respectively[25,26]. The factor 0.6049 was used for $^{147}Sm/^{144}Nd$ (ref. 27).

† Errors are $2\sigma_m$.

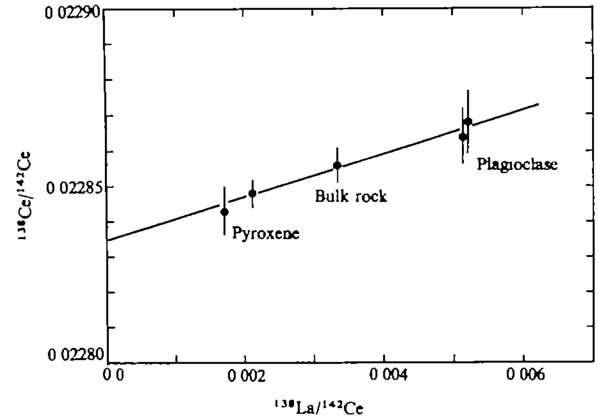

Fig. 1 La–Ce mineral isochron plot for an upper zone gabbro of the Bushveld complex, South Africa. The isochron yields an age of 2,390±480 (2σ) Myr with an initial ^{138}Ce/^{142}Ce ratio of 0.022835±0.000004 (2σ) (λ^{138}_{β} La = 2.58×10^{-12} yr^{-1}).

by G. W. Lugmair) was used as a reference standard. Our ^{143}Nd/^{144}Nd ratio was 0.51183±0.00003 (2σ). All seven Nd isotopes for samples were measured to check the reliability of ^{143}Nd/^{144}Nd measurement. Most ^{142}Nd/^{144}Nd and ^{145}Nd/^{144}Nd ratios agreed with those of the standard reagent within $\pm1\varepsilon$, and ^{148}Nd/^{144}Nd and ^{150}Nd/^{144}Nd ratios agreed with the standard within $\pm2\varepsilon$. A Micromass 30–54R was used for Ce and Nd measurement.

Results are shown on Table 2 and in Figs 1 and 2. The isochron lines were fitted using the York method[12]. Uncertainties (2σ) for ^{138}La/^{142}Ce and ^{147}Sm/^{144}Nd were estimated at 1% and 0.5% respectively. Assuming a partial half life of 2.95×10^{11} yr ($\lambda_\beta = 2.35\times10^{-12}$ yr^{-1}) for ^{138}La (Table 1), the La–Ce isochron yields an age of 2,630±530 Myr (2σ). Similarly, using the modified partial half life of 2.69×10^{11} yr ($\lambda_\beta = 2.58\times10^{-12}$ yr^{-1}) in Table 1 yields an age of 2,390±480 Myr. The former age, 2,630±530 Myr, seems slightly older than the Sm–Nd age (2,050±90 Myr (2σ) with an initial ^{143}Nd/^{144}Nd ratio of 0.50964±0.00010(2σ)); the latter age, 2,390±480 Myr obtained with the modified decay constant is in better agreement with the Sm–Nd age. This suggests that the absorbed H_2O and CO_2 correction in the decay constant measurement as discussed above, was pertinent and that the modified decay constant in Table 1 is more appropriate.

The Bushveld mafic phase has been studied by several investigators. Ages cited here were recalculated on the basis of the new decay constants[13]. Separated biotite from a gabbro of Rustenburg Plats gave a Rb–Sr age of 2,007±49 Myr (2σ) (ref 14). Hamilton[15] determined the Rb–Sr whole rock ages systematically from lower zone to upper zone. These ages are in reasonable agreement through the layer of the mafic rock phase, ranging from 1,901±16(2σ) to 2,126±61(2σ) Myr. A biotite–plagioclase–whole rock internal isochron age, 2,148±14 Myr, is in agreement with the whole-rock isochron ages. Hamilton averaged three selected isochron ages, that is 2,091±94 Myr from the Upper Zone, 2,095±14 Myr from the Merensky Reef and 2,035±18 Myr from the Transition Zone, and presented an age of 2,050±23 Myr as the most reliable for the Bushveld mafic phase. La–Ce and Sm–Nd ages agree with published Rb–Sr ages, although the experimental uncertainty of La–Ce age is considerable.

The ^{143}Nd/^{144}Nd ratio of chondritic uniform reservoir (CHUR) at 2,050 Myr ago was calculated to be 0.51002 by using ^{143}Nd/^{144}Nd ratio (0.50682) of the achondrite Angra dos Reis[16], Sm/Nd ratio (0.321 in weight)[17] of Leedey chondrite and the decay constant (6.54×10^{-12} yr^{-1}) of ^{147}Sm. The initial ^{143}Nd/^{144}Nd ratio, 0.50964±0.00010 (2σ), of the Bushveld upper zone gabbro is slightly lower than the value of the CHUR at 2,050 Myr ago. This fact suggests that the gabbro was derived from a slightly light REE (also probably large ionic lithophiles (LIL)) enriched source compared with CHUR. This is consistent with the Sr data. Two initial ^{87}Sr/^{86}Sr ratios for the upper zone gabbro were obtained as 0.70769±0.00006 (2σ) and 0.70735±0.00008 (2σ) (ref.15). These ratios suggest that the gabbro was derived from LIL enriched source as deduced from the ^{143}Nd/^{144}Nd ratio. Coherent variation of La–Ce and Sm–Nd as a rare earth group suggests that the initial ^{138}Ce/^{142}Ce ratio ($R^{\rm Ce}_{\rm gabbro}$ (2,390 Myr)), 0.022835±0.000004 (2σ), must be higher than the ^{138}Ce/^{142}Ce of CHUR ($R^{\rm Ce}_{\rm CHUR}$ (2,390 Myr)) at that time.

Our results confirm that the La–Ce isotopic system is a promising new geochronometer. Moreover, the La–Ce system, coupled with the Sm–Nd system, will become a unique tool for isotope geochemistry, because both pairs belong to a rare earth group, and this is expected to throw a new light on those studies. In the following section, we will show the variation of ^{138}Ce/^{142}Ce for some terrestrial materials and meteorites.

As discussed above, the gabbro is considered to be derived from a slightly more radiogenic source on ^{138}Ce than CHUR; $R^{\rm Ce}_{\rm gabbro}$ (2,390 Myr) > $R^{\rm Ce}_{\rm CHUR}$ (2,390 Myr).However, it is reasonable to assume that Ce in the source of the gabbro was less radiogenic than the continental crust. Then, $R^{\rm Ce}_{\rm CHUR}$ (4,550 Myr) should be lower than the value obtained by tracing back the $R^{\rm Ce}_{\rm gabbro}$ (2,390 Myr) to T = 4,550 Myr by retrograde decay correction with assuming chondritic La/Ce ratio (0.387 by weight), while it should be higher than the value obtained by tracing back the $R^{\rm Ce}_{\rm gabbro}$ (2,390 Myr) to T = 4,550 Myr by retrograde decay correction assuming the crustal La/Ce ratio (the value of North American Shale, 0.457 was used)[18]. Then, $R^{\rm Ce}_{\rm CHUR}$ (4, 550 Myr) is calculated to be higher than 0.022814 and lower than 0.022817 by using λ^{138}_{β} La = 2.58×10^{-12} yr^{-1}. The La/Ce of the source region of the gabbro is expected to be closer to chondritic than crustal in chemical composition and in the La/Ce ratio. Then we choose rather arbitrarily 0.022816 as a $R^{\rm Ce}_{\rm CHUR}$ (4,550 Myr) between 0.022814 and 0.022817.

The ε notation is used as it is generally on Sr and Nd. ^{138}Ce/^{142}Ce ratios are given as fractionated deviations in parts per 10^4 ($\varepsilon_{\rm Ce}$) from the value in the CHUR and are given by $\varepsilon_{\rm Ce}(T) \equiv [R_{\rm S}(T)/R_{\rm CHUR}(T)-1]\times10^4$ where $R^{\rm Ce}_{\rm S}(T)$ is the ^{138}Ce/^{142}Ce of a sample (s), T is the age under consideration and λ^{138}_{β} La = 2.58×10^{-12} yr^{-1}. $R^{\rm Ce}_{\rm CHUR}$ (0) = 0.022853 is obtained as a value for ^{138}Ce/^{142}Ce in CHUR today (Fig. 3).

Several elements of REE in Isua gneisses were determined[19]. The lanthanum that was not analysed was estimated by extrapolation of Ce–Nd line on chondrite-normalized REE pattern.

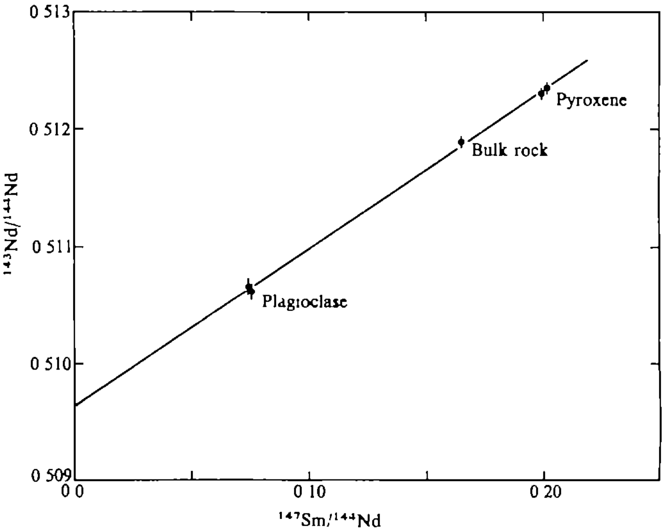

Fig. 2 Sm–Nd mineral isochron plot obtained from an aliquot of the same sample solution as that for La–Ce analysis. The isochron yields an age of 2,050±90 (2σ) Myr with an initial ^{143}Nd/^{144}Nd ratio of 0.50964±0.00010 (2σ) (λ^{147}_{α} Sm = 6.54×10^{-12} yr^{-1}).

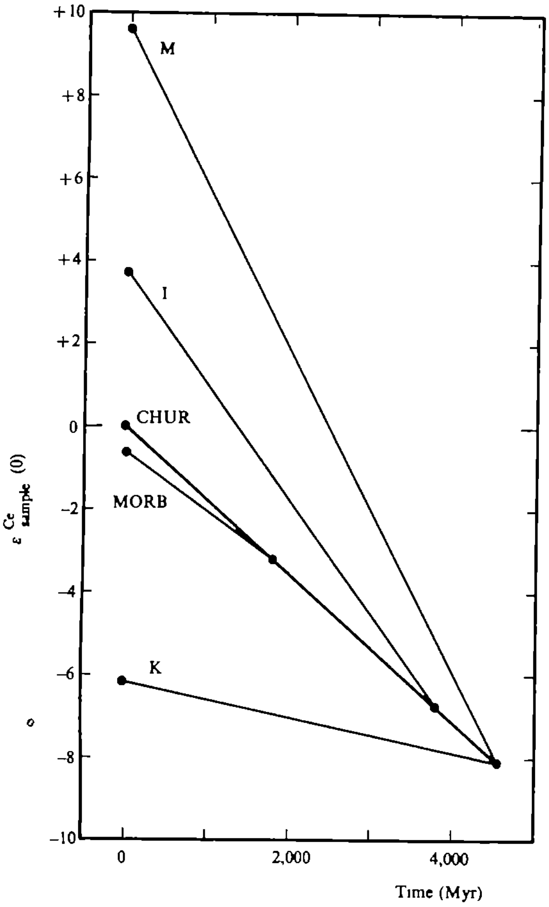

Fig. 3 Calculated ^{138}Ce/^{142}Ce growth curve; Melrose-b howardite (M), Isua gneiss (I), chondritic uniform reservoir (CHUR), mid-oceanic ridge basalt (MORB) and Khohar chondrite (K). Ordinate indicates $\varepsilon_{Ce}(0)$ scale, that is relative to CHUR at present, 0.022853.

$(La/Ce)_{weight} = 0.526$ was obtained for Isua granodiorite gneiss 155766. If we assume that the gneiss was formed from CHUR at 3,770 Myr ago[20], $\varepsilon_{Ce}(0)$ of the gneiss is obtained to be +5.3 (Fig. 3). As discussed from Sm–Nd isotopic systematics by Masuda and Dohmoto[21], if mid-oceanic ridge basalts (MORB) with $(La/Ce)_{weight} = 0.318$ is assumed to be formed at 1,800 Myr ago, $\varepsilon_{Ce}(0)$ of MORB is calculated to be −1.3 (Fig.3). There should be a negative correlation between ε_{Ce} and ε_{Nd}.

For some meteorites with positive or negative Ce anomalies, such as Khohar (L3) and Melrose-b(howardite), larger variations of ^{138}Ce are expected. Khohar$(La/Ce_{weight} = 0.0911)$[22] and Melrose-b$(La/Ce_{weight} = 0.846)$[23] are considered to have $\varepsilon_{Ce}(0)$ of −12.3 and +19.3, respectively, under the assumption that those La/Ce variations were formed at 4,550 Myr ago (Fig. 3). A hibonite inclusion in Allende (HAL) has a La/Ce weight ratio higher than 103 (ref. 24). It will have $\varepsilon_{Ce}(0)$ of more than 4,301.

We conclude that La–Ce isotopic system is a promising geochronometer and also will be a unique tool for isotope geochemistry. It is now essential to establish a reliable new value for the decay constant of ^{138}La.

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ESR dating of planktonic foraminifera

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Isotopic and palaeontological studies on Quaternary deep-sea sediment have provided relative but well-established time scales with ages mostly calibrated from the magnetic-polarity time scale. Absolute dating techniques such as U-series or K/Ar-dating have also been successful; however, difficulties exist such as elemental concentrations near the detection limit, or a lack of the necessary minerals. I report here on electron spin resonance (ESR) dating of marine cores, which utilizes the damage caused by natural radiation in planktonic foraminifera. An ESR signal grows linearly with the γ irradiation dose. It is found that the total radiation doses in the foraminifera increase with depth in two cores studied. The results demonstrate the viability of ESR dating for planktonic foraminifera up to 1 Myr in age.

The results presented here are for planktonic foraminifera of diameter 250–503 μm from deep-sea cores KH 73-4-7 and KN 73-4-8 (from the Melanesia Basin of the western equatorial Pacific Ocean, at 2° 41′ N, 164° 50′ E, water depth 4,160 m and 1° 33′ S, 167° 39′ E, water depth 4,000 m, respectively). ESR spectra of 150 mg samples were measured with an JES-ME2X ESR-spectrometer at room temperature. Core sediments were dated in units about 8 cm long. Artificial irradiation from a ^{60}Co source (dose rate of 10^4 rad h^{-1}) was performed at the Radiation Institute of Science and Industrial Research, Osaka University.

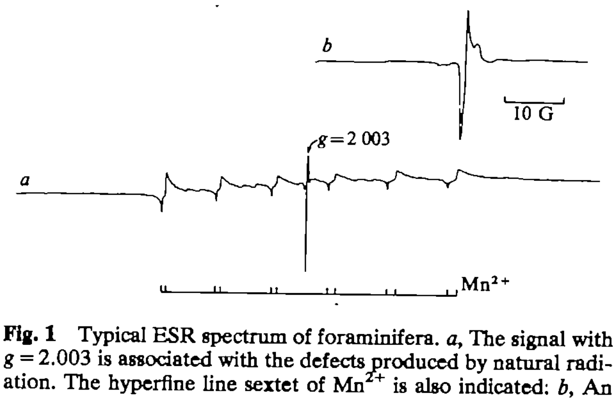

Fig. 1 Typical ESR spectrum of foraminifera. *a*, The signal with $g = 2.003$ is associated with the defects produced by natural radiation. The hyperfine line sextet of Mn^{2+} is also indicated: *b*, An expanded spectrum of the signal associated with the defects.

Table 1 Remarkable levels in two cores and ESR data

		KH 73-4-7				KH 73-4-8			
Levels	Ages (Myr)	Depths (cm)	Mean sedimentation rates (m Myr^{-1})	Mean annual doses (rad yr^{-1})	ESR ages (Myr)	Depths (cm)	Mean sedimentation rates (m Myr^{-1})	Mean annual doses (rad yr^{-1})	ESR ages (Myr)
Lower Olduvai	1.87[13]	884[6]	—			884 *	—		
boundary			4.0				0.6		
Upper Olduvai	1.67[13]	805[6]	—			873 *	—		
boundary			5.7				2.9		
Lower Jaramillo	0.97[13]	407[5]	—	0.102	1.02	670[5]	—	0.113	0.93
boundary			7.0				6.9		
Upper Jaramillo	0.90[13]	358[5]	—	0100	0.92	622[5]	—	0.116	0.88
boundary			5.7				5.2		
Brunhes–Matuyama	0.73[13]	261[4]	—	0.097	0.73	533[5]	—	0.128	0.73
boundary					(Fixed)				(Fixed)
Correspondence of palaeomagnetic intensity (lower)		145 *	5.3		0.50	280 *	13.8		0.52
P. lacunosa, Last appearance	0.474[11]	125[8]	—	0.096	0.46	180[8]	—	0.107	0.42
Correspondence of palaeomagnetic intensity (upper)		40 *	2.6		0.30	110 *	3.8		0.34
E. huxleyi, First appearance	0.275[11]	15–22 †				71–80 †			0.31
Acme Zone	0.07[14]								

The mean annual doses are estimated from the TDs shown in Fig. 3 and the reported ages for the levels. The ESR ages are estimated from the TDs shown in Fig. 3 and the mean annual dose at the Brunhes–Matuyama boundary; a constant annual dose assumed is arbitrarily set equal to the mean annual dose at the boundary for each core.

* Results on palaeomagnetic field intensity, which will be reported elsewhere.

† S. Nishida, personal communication.

Figure 1 shows a typical ESR derivative line of the foraminifera, with a predominant signal associated with the radicals created by natural radiation and the hyperfine line sextet of Mn^{2+}.

The relative intensity of the radical signal is enhanced by exposure to ^{60}Co γ rays (Fig. 2). The total dose (TD) was determined by the additive dose method, in which the signal intensity of the samples is compared with that of identical samples which have also received a laboratory radiation dose. This method is used in thermoluminescence dating[1,2] and ESR dating[3], where the TD is frequently termed the AD (archaeological dose). To minimize errors caused by changes in instrument sensitivity and differences in sample weight, the signal intensity of the irradiated samples was measured relative to the ratio of the intensity of Mn^{2+} signals in the irradiated samples to the unirradiated ones, the Mn^{2+} signals were used as an internal standard. As the concentration of Mn^{2+} is high, the change in the signal intensity of Mn^{2+} by irradiation in this dose range is negligible. Growth of the signal intensity is almost linear up to ~250 krad. Maximum irradiation doses here are below 200 krad for samples which were expected to show high TD.

Figure 3 shows TDs obtained from the results shown in Fig. 2 as a function of core depth. The TDs seem to show an exponential increase towards a saturation level for each core. However, palaeomagnetic and biostratigraphical studies demonstrate that the mean sedimentation rate of the past 0.47 Myr is less for each core than in earlier times (Table 1): the Brunhes–Matuyama boundary, the Jaramillo event and the Olduvai event were observed for each core[4–6]. A microbiostratigraphical investigation of the cores studied by Takayanagi *et al.*[7] showed a level of the last appearance of *Pseudoemiliania lacunosa*, which presumably occurred within isotope Stage 12 (~0.474 Myr)[8]. The small TDs at the uppermost portion are associated with the low sedimentation rate. Therefore the TDs should be considered to increase linearly with depth until the Jaramillo event as shown in Fig. 3. Note that the TDs estimated from the linear increase for the remarkable levels are almost proportional to the reported ages in the levels in both cores, especially in KH 73-4-7. The mean annual dose is almost constant for each core as shown in Table 1.

The K-, U- and Th- contents within a deep-sea core[9,10] seem to fluctuate rapidly with depth. The monotonic increase in the TD with depth may be interpreted as being the influence of variation in the contents smoothed out by penetration of the γ rays over 30 cm.

There are three possible causes of the extremely small TDs observed near the surface. First, changes can occur in the sedimentation rate due to dissolution of calcium carbonate, and deposition of really young sediment, or second, in the annual dose near the surface of the sea bottom. Wintle and Huntley[11] pointed out that the sediment within 30 cm below the sediment–water interface does not receive the full γ dose; therefore, it is possible that after a period of deposition at a constant rate, deposition may have ceased since ~0.2 Myr. Third, changes can occur in the annual dose due to the exponential decrease of the ^{230}Th excess. In this case, the mean annual dose should also decrease but more gradually. The almost constant mean annual doses shown in Table 1 indicate little influence of the ^{230}Th excess. A microbiostratigraphical study of the uppermost 1 m of the core (S. Nishida, personal communication) shows evidence of dissolution of calcium carbonate. Species which have thin and fine structures in a coccosphere were not observed and the species assemblage was simplified in both cores. A level of the first appearance of *Emiliania huxleyi*, which presumably occurred within isotope Stage 8 (~0.275 Myr)[8], was observed by Nishida between 15 and 22 cm for KH 73-4-7. However, this portion may correspond to the beginning of the acme zone of the species, as the coccosphere is rather sensitive to dissolution. On the other hand, in KH 73-4-8 the level where the species first appeared was indicated between 71 and 80 cm, however, the species was not observed in portions of this core shallower than 31 cm. The disappearance of the species is probably due to dissolution and the fact that part of the acme zone could not be collected. We assume therefore that the small

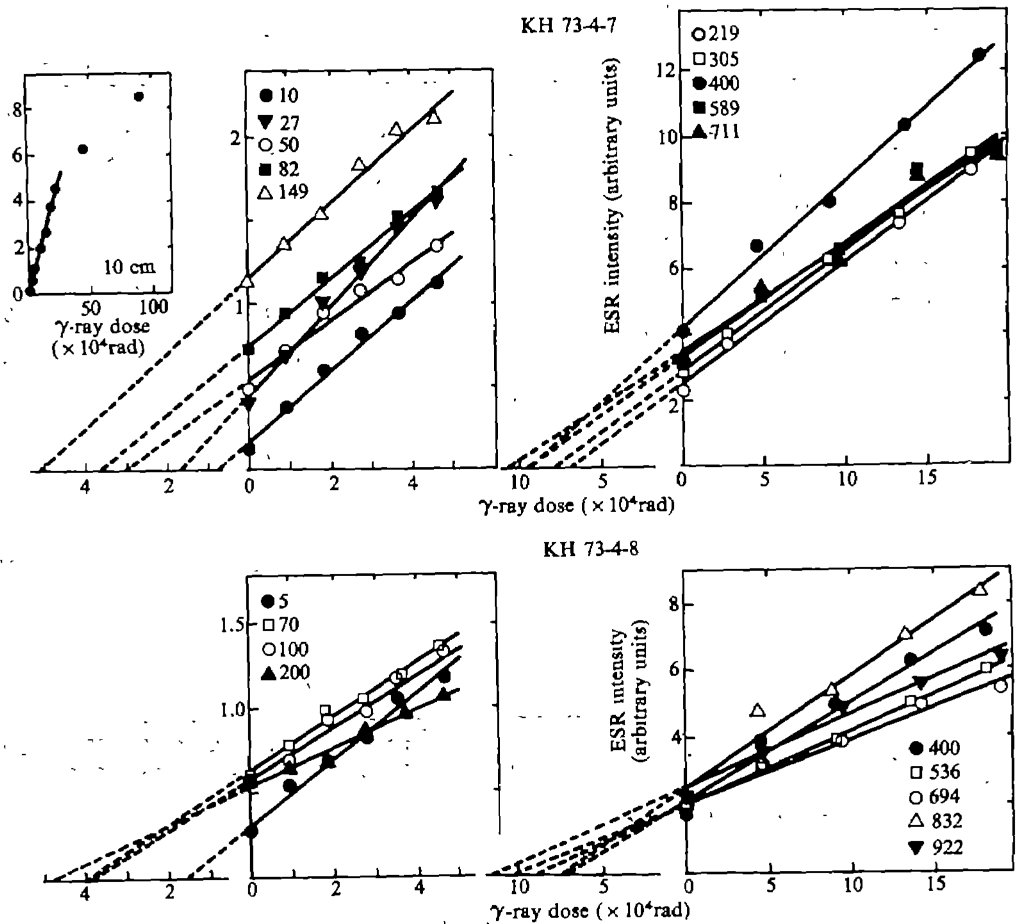

Fig. 2 Growth of the ESR signal of foraminifera from two cores with artificial γ irradiation. The numbers indicate the depths of each core.

TDs at the shallower depth are due to dissolution of calcium carbonate.

Samples taken from portions deeper than those corresponding to the Jaramillo event in both cores did not show any further linear increase of TDs with either depth or age, although the accuracy of TDs decreases. Wintle[12] reported that the mean lifetime of the 275°C TL peak of calcite was 390 Myr at 5°C, and that the anomalous fading, the effect of which could not be detected by mean lifetime estimation, had been observed for a sample of labradorite feldspar. As the tendency towards saturation was observed downward from the portion of similar ages in two cores with different depositional environments, it may be caused by the anomalous fading in calcite.

The ESR ages, determined on the basis of the ages of the Brunhes–Matuyama boundary, assuming a constant annual dose for each core, are compared in Table 1. Detailed palaeomagnetic investigation of these cores, in which variations in the palaeomagnetic field intensity were deduced from the ratio of natural remanent magnetization to the saturation isothermal remanent magnetization, showed correspondence between the curves of the ratio. The ESR ages of two distinctly corresponding depths are compared in Table 1. The results of the palaeomagnetic investigation will be described in detail elsewhere.

The disagreement between the reported ages and the ESR ages in KH 73-4-7 can be explained by dissolution to the order of 10% in the portion shallower than 2 m. On the other hand, the complicated changes in the sedimentation rate may be associated with the disagreement in KH 73-4-8. These changes lead to errors in the TDs estimated from linear increase. The TDs can be said to be almost proportional to the ages for a core with a constant sedimentation rate.

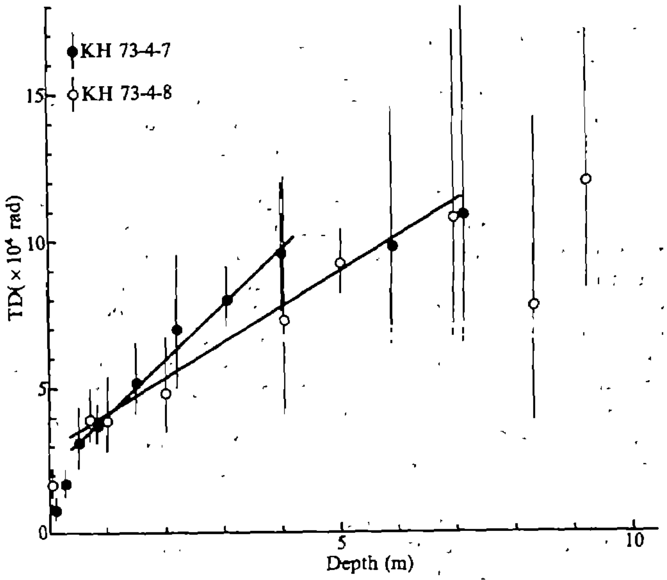

Fig. 3 Total dose (TD) plotted against depth for two cores. The bars represent the 90% confidence level in the TD measurement only.

I conclude that ESR is useful for dating planktonic foraminifera up to 1-Myr old but to obtain detailed changes in the sedimentation rates, some problems must be resolved. More accurate estimation of TD and evaluation of variations in the annual dose versus depth on the basis of the contents of radioactive elements and isotopes are necessary.

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Female mating preference is genetic

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The objection is often raised against sociobiological theory that different behavioural strategies have never been shown to be genetically determined. Yet if they are to evolve at all, the differences must be in part hereditary. Nobody would dispute that many aspects of behaviour—the song of *Drosophila* or birds, or the tendency to migrate, for example—would show genetic variation. But sociobiologists assert that different strategies of behaviour— the alternative courses of action that an animal might take—are also inherited and can thus be selected: strategies for example, such as whether to be a 'hawk' or a 'dove' in conflicts, or whether to choose one type of male because he may be a better mate than others. No doubt there are many aspects of behaviour that vary genetically and thus produce variations in the chances of success in conflicts or in finding mates. But does an animal prefer to follow one course of action rather than other alternative courses, and is its preference hereditary? This is the crucial question for sociobiology. As dogmatically as some sociobiologists have answered 'Yes', so have some population geneticists answered 'No'. Lewontin[1] called Dawkins' *The Selfish Gene*[2] a caricature of Darwinism for assuming that all behaviour may be inherited and natural selection can therefore do anything. These extreme views persist for lack of experimental evidence. We show here that female mating preference can be substantially increased in successive generations by artificial selection. It must therefore have a genetic component.

In *The Descent of Man*, Darwin[3] invoked female preference as one of the two selective mechanisms for the evolution of male characteristics of sexual display. The existence of female preference was a premise of Darwin's theory. Fisher[4] explained its existence as the outcome of an evolutionary process in which both preference and preferred character evolve together. O'Donald[5,6] and Lande[7] analysed models of female preferences that support Fisher's theory. Models of preferential mating have been fitted to observations on the relative frequencies of matings of different genotypes[6], but experiments on the sexual selection of preference have never been carried out over a number of generations.

Preferential mating may be involved in the maintenance of the colour polymorphism of the two-spot ladybird, *Adalia bipunctata*[8,9]. Majerus *et al.*[10] carried out a series of mating choice experiments on melanic (*quadrimaculata*) and non-melanic (*typica*) ladybirds collected at Keele in Staffordshire. *Quadrimaculata* is inherited as a unifactorial dominant to *typica*[11]. The results give a good fit to a model of mixed sexual selection and assortative mating for dominant and recessive traits[12]. The maximum likelihood estimates generated by the model show a strong and very significant non-assorting preference, γ, for *quadrimaculata* males. The assorting preference, c, for these males was appreciable, but not formally significant. Maximum likelihood estimates of assorting and non-assorting preferences for *typica* were very close to zero[10]. Observations of courting pairs indicated that female choice determined the frequencies of the different matings. No male competition for mates has been seen to occur[10].

Following these results, it was obviously imperative to test whether selection will increase the level of female preference in successive generations, and also to confirm experimentally that female choice determines the frequencies of the matings of the different male phenotypes. A sample of ladybirds from the Keele stock was allowed to mate in a population cage. The sample consisted of the following phenotypes: 112♂♂+112♀♀ *typica*, 48♂♂+48♀♀ *quadrimaculata*. This 7:3 ratio of *typica*:*quadrimaculata* was chosen because it gives good estimates of the mating preference at the level at which it occurs in the Keele stock. Our experimental regime has been described elsewhere[10]. We recorded the phenotypes of individuals *in copuli* by continuously observing the cage during a fixed period of 16 h of light per day. We removed the mating pairs of ladybirds. As soon as pairs had separated, we replaced the males in the cage. We placed those females that had mated with *quadrimaculata* males in a new population cage to lay the eggs for the next generation of the selected line. Females which mated with *typica* males were discarded. We repeated this procedure for three subsequent generations, restoring the 7:3 phenotypic ratios at the start of each generation. We also set up a control line, maintained in the same way as the selected line, except that all mating females, regardless of the phenotypes of their mates, laid the eggs of the next generation. The results from both the selected and control lines are given in Table 1. If the deviation from random mating shows an overall preference towards *quadrimaculata* males, then matings take place at the frequencies $\gamma+v(1-\gamma)$ for *quadrimaculata* males, and $(1-v)(1-\gamma)$ for *typica* males, where v is the overall frequency of *quadrimaculata* in the cage and γ the proportion of females expressing a preference for them. Even if the preference is expressed in some partial or frequency-dependent manner, the constancy of v in all experiments ensures that γ will be a constant fraction of the total proportion of females with a preference; hence, γ will always measure the relative magnitude of the preference[6,13]. If n_Q and n_T are the observed numbers of the matings with each phenotype of male, the level of preference is estimated by

$$\hat{\gamma}=\frac{n_Q(1-v)-n_T v}{n(1-v)}$$

where $n=n_Q+n_T$. This maximum likelihood estimate has been derived from a model with no preference for *typica*. Table 1 shows the estimates of preference against the data from which they were obtained. In generation 3, we also set up direct tests of female choice in mating chambers, using progeny from the previous generation and equal phenotypic ratios. In these experiments, each mating pair is immediately replaced by fresh individuals with the same phenotypes as those that had mated. We tested female choice by similar experiments on the original unselected stock. Table 2 shows the results of these mating test experiments.

To differentiate conclusively between male competition and female choice, we set up three further population cage experiments. A sample consisting of males from the progeny of the fourth generation of the selected line and females from the unselected Keele stock were allowed to mate in a population cage. In a second cage, females from the fourth generation of the selected line were allowed to mate with males from the unselected Keele stock. In a third cage, as a control, males and females both taken from the unselected Keele stock were allowed to mate. In all three experiments, 7:3 ratios of *typica*:*quadrimaculata* phenotypes were maintained in the samples. Table 3 gives the frequencies of matings, estimates of preference and statistical analysis.

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Table 1 Numbers of ladybirds in population cages in successive generations and estimates of female preference

Generation	Total nos in cage N_T	N_Q	Q×Q	Nos of matings between different phenotypes Q×T	T×Q	T×T	Estimates of preference for Q $\hat{\gamma} \pm \sqrt{\mathrm{var}(\hat{\gamma})}$
Selected line							
1	224	96	21	31	24	46	0.1803±0.0640
2	112	48	13	18	9	14	0.3915±0.0961
3	84	36	12	14	6	10	0.4558±0.1070
4	140	60	18	21	5	12	0.5663±0.0878
Control line							
1	98	42	13	15	8	21	0.2732±0.0946
2	70	30	7	11	7	12	0.2664±0.1174
3	112	48	10	18	10	18	0.2857±0.0955
4	112	48	11	22	13	28	0.2085±0.0825

Analysis of χ^2

Component of variation	Values of χ^2	d.fs	Values of P
Between selected and unselected groups	12.5103	1	0.000405
Within selected populations	1.6837	2	0.431
Within unselected populations	1.3000	4	0.861

N_T and N_Q refer to the total number of *typica* (T) and *quadrimaculata* (Q) ladybirds of a given generation that were placed in a population cage for mating. They consisted of equal numbers of males and females. The male phenotype is shown first in the type of mating: Q×T is a mating of Q♂×T♀; T×Q is a mating of T♂×Q♀. The males were replaced in the cage after they had mated. The preferred males often mated more than once during the 5 or 6 days of observation of a cage. Females were not replaced after mating. If observations had been continued until all females had mated, an exact 7:3 ratio of T:Q females would necessarily have been found to have mated. However, after 6 days, 90% of Q and 60% of T females had mated. Comparing numbers of mated and unmated females among Q and T females in the selected line, we obtain $\chi_3^2 = 1.4962$, showing no change in mating propensities of Q and T females with increasing preference over generations. The Q and T females show similar levels of preference, but the T females are slower to mate. 'd.fs' in the analysis of χ^2 means the numbers of degrees of freedom.

Table 1 shows that the proportion of females preferring *quadrimaculata* males increased significantly in the selected line but not in the control line. Although obviously decisive, these results present a problem of statistical analysis. A linear increase in preference is not to be expected because the selection differential declines as more females exercise the preference: sexual selection by female choice is necessarily frequency-dependent[6,14]. We should expect that the rate of increase of preference would fall rapidly in successive generations; how rapidly would depend on the genetics of preference which is unknown. A simple linear regression of the increase of γ would be an inappropriate model to fit to the data. The data of both selected and unselected lines form a 2×8 table of numbers of matings of *quadrimaculata* and *typica* males. Generations 2, 3 and 4 of the selected line represent generations after females have been selected for increased preference. These generations can be tested against the unselected generations. Of course, this test ignores the increase within the selected line. It represents a conservative test which should not overestimate the true level of significance.

The data also show that the response of the selected line differs significantly from that of the control line. This is the crucial test. In the absence of an appropriate regression model, a conservative test of the difference in the responses can be constructed by comparing the differences between the first and fourth generations in the selected and control lines. We expect to find a response over the four generations in the selected line but not in the control line. Thus, we ask: do the selected and control lines differ in their responses? The data form a 2^3 table as follows:

	Selected line Q♂♂	T♂♂	Control line Q♂♂	T♂♂
Generation 1	52	70	28	29
Generation 4	39	17	33	41

The difference in Q:T ratio between selected and control lines differs significantly between generations. For this three-factor interaction (phenotypes × lines × generations): $\chi_1^2 = 6.2678$, $P = 0.0123$. Because we have omitted the data of generations 2 and 3, this is a very conservative test which must underestimate the true level of significance in the total data. The difference between selected and control lines in generation 4 is tested by $\chi_1^2 = 8.0939$ (or 7.1120 with Yates' correction), $P = 0.00444$ (or 0.00766 for χ^2 with Yates' correction). The preference has been raised very significantly in the selected line but not in the control line. As shown in Table 2, the difference between selected and control lines in generation 3 is nearly significant in spite of the very small numbers of matings observed for this generation in the selected and control lines. By Fisher's combination of probabilities test applied to the results of generation 3 and 4, we obtain $\chi_4^2 = 14.9761$, $P = 0.00475$, giving the overall significance of the difference between the lines.

Table 2 Numbers of matings of ladybirds in mating choice experiments and estimates of female preference

Q×Q	Nos of matings between different phenotypes Q×T	T×Q	T×T	Estimate of preference for Q $\hat{\gamma} \pm \sqrt{\mathrm{var}(\hat{\gamma})}$
Mating tests on original unselected stock				
72	67	41	46	0.2301±0.0522
Mating tests on generation 3 of selected line				
15	15	6	4	0.5±0.1369

Overall estimates of preference for generation 3

Preference in selected line in generation 3 estimated from data of mating tests and population cage experiments:	$\hat{\gamma} = 0.4721 \pm 0.0846$
Preference in control line in generation 3:	$\hat{\gamma} = 0.2857 \pm 0.0955$
Test of significance of difference in estimates for control and selected lines:	$\chi_1^2 = 3.2120$; $P = 0.0731$

In these mating test experiments, five males and five females of each phenotype are placed in a mating chamber and kept under continuous observation. When a mating takes place, the mating pair are removed and fresh individuals of the same phenotypes are replaced. Both male and female phenotypes are replaced. A strict 5:5 ratio of Q:T males and females is thus maintained in the mating chamber.

Table 3 Mating tests between the selected line and original Keele stock

Nos of matings and estimates of preferences

Stocks and sexes used in matings	Q×Q	Nos of matings between different phenotypes Q×T	T×Q	T×T	Estimates of preference for Q $\hat{\gamma} \pm \sqrt{\mathrm{var}(\hat{\gamma})}$
Selected ♂♂ × Keele ♀♀	11	28	17	34	0.1905 ± 0.0746
Selected ♀♀ × Keele ♂♂	16	28	6	15	0.5385 ± 0.0829
Keele ♂♂ × Keele ♀♀	18	35	21	42	0.2241 ± 0.0661

Analysis of χ^2

Component of variation	Value of χ^2	d.fs	Value of P
Selected males compared with Keele males	0.1126	1	0.737
Selected females compared with Keele females	10.4844	1	0.00120

The selected males and females used in these tests were taken from the progeny of the fourth generation of the selected line. No further selection had been imposed after the fourth generation. The level of preference among the progeny of the fourth generation should be the same as that in the fourth generation. The selected males and females were tested in matings with progeny taken from the corresponding generation of the original Keele stock. Population cages were set up with 7:3 ratios of T:Q. They were kept under continuous observation; mating pairs were counted giving the numbers shown in the table.

The response to selection that we have demonstrated has taken place in the females' mating preference for *quadrimaculata* males. As shown in Table 3, when selected females are mated with males from the original stock, the level of preference is characteristic of the selected line; when selected males are mated with females from the original stock, the level of preference is characteristic of the original stock. Female mating preference has thus been shown to have a large genetic component in its determination. We believe that these experiments provide the first formal proof that a female mating strategy is genetically determined.

This proof is fundamental for theories of the evolution of female preference[4–7]. It shows that female choice of mate can evolve: mate choice will be selected if, as in our experiment, females making the choice contribute a relatively greater proportion of offspring to future generations. In a natural population, they will do so if the males they choose to mate with possess a selective advantage. As the female preference contributes some of this selective advantage, it partly selects itself. This gives rise to what Fisher[4] called the 'runaway process of sexual selection'. Initially, while preference genes are rare—they may perhaps have arisen by mutation—this process will be extremely slow, requiring many thousands of generations to get started[5]. Realistically, male competition or natural selection must provide the initial advantage. The preference will then be selected in the course of the selection of the preferred male character. The dynamics of this selection is very complex and depends crucially on the genetics of the preference[5]. Initially, as a gene for the preferred character spreads through a population, a preference gene may be expected to show a three- to fivefold increase in frequency. As the preference becomes more common, it adds to the preferred males' advantage; but the preference gene itself shows progressively smaller increases in frequency as it becomes more common. The process eventually stops when the male character has developed to the point at which it has become so disadvantageous in natural selection that this disadvantage balances the sexual selection.

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Phenomenal coherence of moving visual patterns

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When a moving grating is viewed through an aperture, only motion orthogonal to its bars is visible, as motion parallel to the bars causes no change in the stimulus. Because there is a family of physical motions of various directions and speeds that appear identical, the motion of the grating is ambiguous. In contrast, when two crossed moving gratings are superimposed, the resulting plaid pattern usually moves unambiguously and predictably. In certain cases, however, two gratings do not combine into a single coherent percept, but appear to slide across one another. We have studied the conditions under which coherence does and does not occur, and we report here that it depends on the relative contrasts, spatial frequencies and directions of motion of the gratings. These effects may reveal the previously unstudied properties of a higher order stage of motion analysis.

Figure 1*a* illustrates the problem of ambiguity[1,2]. The grating moves behind a circular aperture. Any of the physical motions indicated by the arrows will appear the same when viewed through the aperture. The situation can be depicted graphically in 'velocity space', a space in which each vector represents a velocity: the length of a vector corresponds to speed, and its angle corresponds to direction. As shown in Fig. 1*a*, the motion of a single grating is consistent with a family of motions that lies along a line in velocity space; this line is parallel to the grating's bars and orthogonal to the vector representing its 'primary' motion. If two moving gratings are superimposed, the resulting 'plaid' pattern moves with a speed and direction which can be exactly predicted from the velocity space construction: the two loci of possible motions intersect at a single point, corresponding to the motion of the coherent pattern (Fig. 1*b*). This solution to the 'aperture problem' is similar to that proposed (in a rather different context) by Fennema and Thompson[3]. It has advantages over that advanced by Marr and Ullman[4], which can only infer a range of possible directions from the limited information provided by a pair of gratings.

The velocity space combination rule is also different from a vector sum or vector average. In Fig. 1*c*, two gratings move downward and rightward with different speeds and directions.

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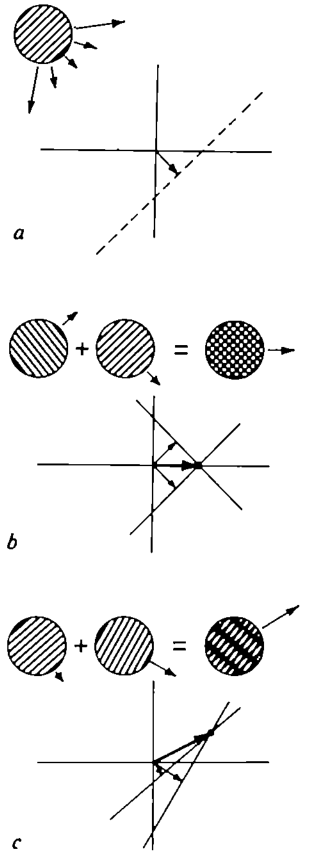

Fig. 1 The velocity-space representation of some moving patterns. In each panel, a vector represents a motion in a direction given by the vector's angle at a speed given by the vector's length. *a*, A single grating moves behind an aperture. The broken line indicates the locus of velocities compatible with the motion of the grating. *b*, A plaid composed of two orthogonal gratings moving at the same speed. The lines give the possible motions of each grating alone. Their intersection is the only shared motion, and corresponds to what is seen. *c*, A plaid composed of two oblique gratings, one moving slowly and the other more rapidly. Both gratings move down and to the right, but the pattern moves up and to the right.

A vector sum or average would predict a pattern motion that was also downward and rightward, but the velocity space solution is a motion upward and rightward, the motion that observers report when the gratings are seen to cohere[5]. Of course, nothing requires that coherent motion be seen, and instead of a single rigidly moving plaid, the two gratings are sometimes seen sliding 'incoherently' over each other. We have studied the stimulus conditions under which coherence occurs, in order to characterize the mechanisms that underly the coherent percept and resolve the ambiguity of one-dimensional motion.

Observers viewed a circular cathode ray tube display subtending 5°; the luminance of the display was constant on average, but was modulated by signals from a PDP11 computer to produce a superimposed pair of sinusoidal gratings that could each be varied in orientation, direction and speed of motion, contrast and spatial frequency. Observers were strictly instructed not to make judgements unless their eyes were steadily fixated on a small black circle in the centre of the display. Subjects under this kind of instruction are capable of stable and reliable fixation[6]. After each 1.5-s exposure, the observer indicated whether the pattern appeared 'coherent' or 'incoherent'.

Contrast strongly affects coherence, even when both gratings are easily visible. Figure 2*a* shows a psychometric function for detection and a similar function for coherence, when the contrast of one of the gratings in a plaid was varied (the other grating's contrast was fixed at 0.3). The gratings were of the same spatial frequency, and moved at an angle of 135° to one another. Open symbols show the probability of detecting the low-contrast grating in the presence of its high-contrast mate. Filled symbols show the probability of a coherent percept. Both detection and coherence become more likely as contrast increases, but reliable coherence only occurs at contrasts where both gratings are clearly visible.

In further experiments, we used this contrast dependence to measure the relative tendency of different pairs of gratings to cohere. We fixed the contrast of one grating at 0.3, and used a staircase procedure to vary the contrast of the other grating until the observer saw coherent motion on half the trials. The gratings' speed, orientation and spatial frequency all affected the strength of coherence. Coherence decreased as the speed of the component gratings increased, as the angle between their 'primary' directions increased, and as the difference between their spatial frequencies increased.

Figure 2*b* shows the effect of relative spatial frequency on coherence. Filled symbols illustrate a case in which the spatial frequency of the high-contrast grating was fixed at 1.2 cycles deg^{-1}, while the spatial frequency and contrast of the second grating were varied; open symbols illustrate a case where the spatial frequency of the first grating was fixed at 2.2 cycles deg^{-1}. As the frequencies of the two gratings were made different, the tendency to cohere was reduced, and the contrast needed for coherence was increased. This spatial frequency dependence suggests that coherent motion, like many other visual properties, is analysed by mechanisms that are selective for spatial frequency.

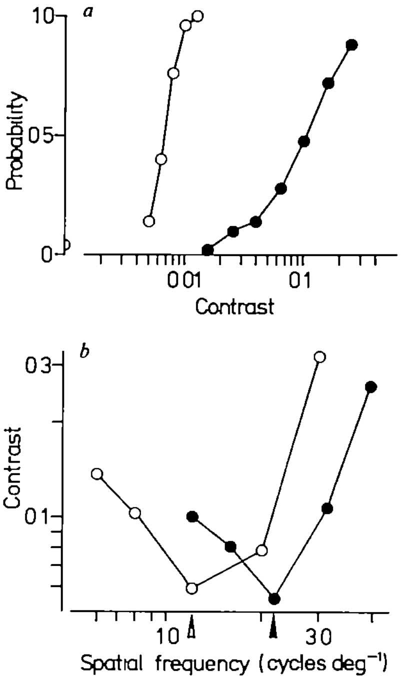

Fig. 2 Two parameters influencing perceptual coherence. *a*, The influence of contrast on the detectability and coherence of two crossed sinusoidal gratings. One grating was fixed in contrast at 0.3. The other was of variable contrast, and moved at an angle of 135° to the first; both had a spatial frequency of 1.6 cycles deg^{-1}, and moved at 3 $\mathrm{deg\,s}^{-1}$. Filled symbols show the probability that the observer judged the two gratings to be 'coherent'; open symbols show the probability that the observer detected the presence of the lower contrast grating. The half-circle on the ordinate shows the probability that the observer judged the second grating to be present when its contrast was zero—the 'false-positive rate' for detection; the false-positive rate for coherence judgments was zero. Subject, E.H.A. *b*, The influence of spatial frequency on coherence. The standard grating again had a contrast of 0.3 and moved at 3 $\mathrm{deg\,s}^{-1}$. The test gratings moved at an angle of 135° to the standard, also at 3 $\mathrm{deg\,s}^{-1}$. Open symbols show the results for a range of test spatial frequencies when the standard grating had a spatial frequency of 1.2 cycles deg^{-1} (open arrow). Filled symbols show the results when the standard grating had a spatial frequency of 2.2 cycles deg^{-1} (filled arrow). Subject, P.A.

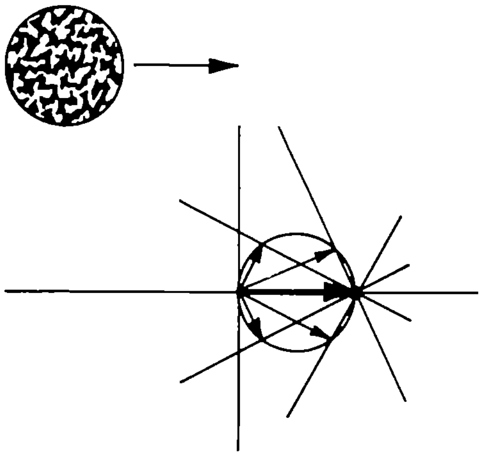

Fig. 3 The velocity-space representation of a random-texture field moving to the right. The field contains components of all orientations, and the primary motion vector for each ends on the circle passing through the origin; the common motion 'implied' is the rightward motion given by the bold arrow. This circular locus of primary motions represents all the motions that can exist in a single rightward-moving pattern. As such, the locus could represent the preferences of a family of primary motion analysers whose outputs are combined to signal coherent two-dimensional motion (see text).

There are two kinds of model that might account for our results. The first relies on the presence in our patterns of localizable blob-like features where the peaks and troughs of the crossed gratings intersect. Some process based on direction-selective elements not selective for orientation might detect and signal the motion of these 'blobs', which is of course identical to the motion of the pattern as a whole. We have evidence to suggest that such a scheme is incorrect. We superimposed one-dimensional dynamic random visual noise (a rapidly changing pattern of random-width lines)[7] on our test patterns. A non-oriented mechanism that responds directly to the motion of blobs should be most affected by noise oriented at right angles to the coherent motion. But a mechanism that combines the outputs of oriented channels should be most affected when those channels are most affected—when the noise is oriented at right angles to the primary motion of one or the other of the component gratings. Our results support the orientation-based model. Coherence is strongly reduced when the noise mask is perpendicular to a component's motion (and thus parallel to the component's orientation), but is almost unaffected when the mask is perpendicular to the coherent motion of the pattern. This suggests that some orientation-selective process must precede the analysis of coherent motion, and our second model incorporates this feature.

There is abundant evidence that visual analysing mechanisms selective for orientation and spatial frequency exist at a relatively early stage in the visual pathway[8–12]. It is clear that such mechanisms are often sensitive to the direction of motion of one-dimensional contours[8,9,13–15], but they also seem unable to signal the true direction of motion of two-dimensional patterns[16–19]. The perceptual coherence of two gratings into a single moving plaid may therefore represent the action of an additional stage of visual processing beyond those usually considered to be involved in analysing motion. At this second stage, the outputs of several 'one-dimensional' motion analysers would be combined. Coherence phenomena may offer an approach to studying the properties of this postulated second-stage motion analyser.

Figure 3 shows how the velocity space construct helps to assign motion to more complex patterns, such as a horizontally moving random-texture field. The texture contains components of many orientations; their motions are represented in velocity space by the arrows in Fig. 3. As the speed of each component is proportional to the cosine of the angle between its direction and horizontal, the vectors all end on a circle that passes through the origin. Note also that the motion 'families' associated with the various components all pass through the common point corresponding to the horizontally directed pattern motion.

A given vector in Fig. 3 could represent the preference of a one-dimensional motion detector, and the circle might represent the 'receptive field' in velocity space of a second-stage motion analyser. If it combined the outputs of one-dimensional analysers corresponding to points on the circle by an operation akin to a logical 'and', the second-stage analyser would respond only to a particular direction of motion of the two-dimensional stimulus. Our results on perceptual coherence might reflect the relative strengths of the different inputs to such a motion analyser. They would suggest that this analyser computes the weighted combination of signals arising from a collection of one-dimensional motion analysers having different velocity preferences but similar spatial frequency preferences.

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Prenatal misrouting of the retinogeniculate pathway in Siamese cats

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In the mammalian visual system, retinal ganglion cells send their axons to several distinct structures in the brain. One of these, the lateral geniculate nucleus (LGN), receives a substantial projection from both eyes in higher mammals such as the cat[1–3]. How does this pattern of input arise during development? One possibility is that retinal ganglion cell axons project from the very beginning to the appropriate side of the brain. Another is that the initial projection may contain many inappropriate connections which are eliminated after the axons reach their target, the LGN. We thought the Siamese cat might provide an opportunity to study these alternatives. Guillery and others[4–7] have shown that in adult Siamese cats, the LGN receives a disproportionately large input from the contralateral eye and a correspondingly diminished input from the ipsilateral eye due to a genetic mutation at the *albino* locus. We report here that this abnormal pattern of input is present from the earliest stages of development of the retinogeniculate projection and arises as a consequence of the misrouting of fibres at the optic chiasm. These findings suggest that during normal development, growing optic fibres can be directed to the appropriate side of the brain.

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In normal adult cats the retinal ganglion cells within each eye are sharply divided into two halves by virtue of their axonal projections to the LGN[8,9]. Figure 1*a* shows that axons from retinal ganglion cells in the nasal half of each eye cross in the optic chiasm and terminate in layers A, C and C2 of the contralateral LGN. Axons from retinal ganglion cells in the temporal half of each eye do not cross but project instead to the ipsilateral LGN where they terminate in the alternate A1 and C1 layers[1,10].

In contrast, in adult Siamese cats the nasotemporal division is abnormal, (see Fig. 1*b*). In these animals the contralateral projection to the LGN consists of axons from retinal ganglion cells in the nasal retina, as usual, plus an abnormal component from the temporal retina which projects to portions of layers A1 (abA1) and C1. A reduced number of the remaining retinal ganglion cells in the temporal half of the retina send axons to the ipsilateral LGN, where they are confined to several small patches in layers A1 (mnA1 and lnA1) and C1[6,7,11,12].

We wished to determine how the abnormal Siamese pattern of the retinogeniculate projection arises during development. Therefore, we labelled the projection in 26 Siamese and 43 normal newborn and fetal cats of known gestational age by using the axonal transport of intra-ocularly injected horseradish peroxidase (HRP) or ^{3}H-leucine. (Siamese fetuses were obtained from breedings within our own pure-bred Siamese colony.) The cat's gestation period averages 65 days, with the first day of breeding designated as embryonic day 0 (E0) and birth as postnatal day 0 (P0). Prenatal eye injections were performed using the fetal surgery techniques of Rakic[13] modified for the cat[14]. In most cases both eyes were injected with different labels. Animals were allowed to survive for 24 h, then removed by caesarean section and processed for HRP histochemistry[14,15] and/or autoradiography[7]. Complete and even labelling of the retinas in all the animals studied here was verified by examining the retinas (autoradiographically or histochemically) and by inspecting the pattern of the retinal projections to the LGN and superior colliculus. At each age the labelling pattern was considered to reflect accurately the retinogeniculate projection only when similar results were obtained from three or more injections.

By birth the pattern of axonal projection from retina to LGN as revealed autoradiographically or histochemically is adult-like, both in Siamese and normal cats—see top of Fig. 2, which shows a matched series of darkfield photographs of peroxidase- or radioactively-labelled sections through the LGNs of progressively younger normal and Siamese cats. At postnatal day 2, comparison of Fig. 2*a* (normal) and *f* (Siamese) demonstrates the drastically reduced ipsilateral and correspondingly increased contralateral projections that are typical of the retinogeniculate projection in adult Siamese cats. Compare also Fig. 2*f* with Fig. 1*b*—what remains of the ipsilateral projection in Fig. 2*f* is confined to several small labelled patches.

We next made a prenatal study. The left-hand side of Fig. 2 shows the general time course and pattern of development of the retinogeniculate projection in a series of normal cat fetuses. By E32, a clear projection from the retina to the lateral margin of the thalamus is present bilaterally, with that from the contralateral eye being more extensive than that from the ipsilateral eye (Fig. 2*e*). Note that at this age the label is largely restricted to the optic tracts, particularly ipsilaterally. At older ages (E35, E40; Fig. 2*d*, *c*, respectively), the ipsilateral and contralateral projections increase, and occupy progressively more medial territory within the LGN. By E54 (Fig. 2*b*), afferents from the two eyes are in the process of segregating from each other to form the set of sharply defined layers present at birth (Fig. 2*a*). (For fuller accounts of normal development, see refs 14, 16.)

The overall developmental pattern of the retinogeniculate projection in Siamese cats shown on the right-hand side of Fig. 2 differs from that of normal animals in one important respect; at each embryonic age there is a striking reduction in the size of the ipsilateral projection and a relative increase on the contralateral side. This size disparity is most apparent at E51 (Fig. 2*g*) when, as in normal animals, the retinogeniculate afferents from the two eyes are in the process of segregating from each other. In the Siamese fetus, however, the result is the appearance of the characteristic abnormal adult-like pattern (compare Fig. 2*f* and *g*). Earlier, at E46 (not shown), the projection pattern is similar to that seen at E51 in that the projection to the ipsilateral LGN remains significantly smaller in size and extent than that to the contralateral side. At E41, when the contralateral projection to the LGN is already well established, only a small contingent of ipsilateral fibres have invaded the posterior pole (Fig. 2*h*, arrow). Most of the remaining ipsilateral label is confined to the optic tract and brachium of the superior colliculus. Before E41, ipsilateral label is almost entirely restricted to the tract in the Siamese fetuses. This is the case at E36 (Fig. 2*i*), when the LGN is invaded normally by the first ipsilateral retinal fibres (Fig. 2*d*, arrow). Indeed, as early as E32, when the retinogeniculate afferents initially arrive within the vicinity of the LGN anlage, but in normal animals are still confined largely within the optic tract (Fig. 2*e*), the ipsilateral projection is absent from the Siamese fetus (Fig. 2*j*).

These results indicate that the LGN receives a diminished ipsilateral retinal projection throughout fetal development in Siamese cats. It is worth noting here that in the development of retinogeniculate connections in *albino* rats, Lund and coworkers[17] found that the diminished ipsilateral projection emerges only secondarily, largely due to a massive elimination of connections once they had reached the LGN. Such differences between the two species may simply reflect differences in the action of the mutant gene. Alternatively, they may represent significant differences in the mechanisms underlying normal development—mechanisms that in higher mammals, but not in rodents, operate to produce a large and precise ipsilateral projection.

At least two distinctly different events could account for this reduction in the size of the ipsilateral projection within the

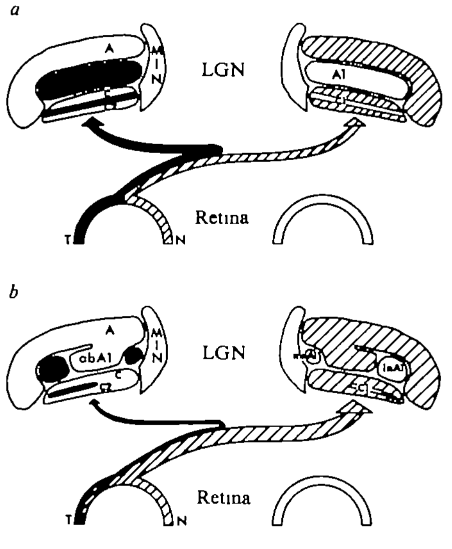

Fig. 1 A diagram of the retinogeniculate projection in normal (*a*) and Siamese (*b*) adult cats. Only connections between the left eye and the LGN are shown here for simplicity. *a*, Normally, axons from retinal ganglion cells in the nasal half of each retina (hatched area labelled N) cross at the chiasm and project to layers A, C and C2 of the contralateral LGN. Those in the temporal half (black area labelled T) project ipsilaterally to the alternate A1 and C1 layers. *b*, In the Siamese cat, the LGN receives an abnormally large projection from the contralateral retina (right LGN: hatched area), consisting of additional fibres from the temporal retina (hatched area) that innervate portions of layers A1 (abA1) and C1. There is a correspondingly reduced projection from the ipsilateral retina (left LGN: black areas) that terminates in several small patches medially (mnA1) and laterally (lnA1) in layer A1, and in layer C1. The projection to the medial interlaminar nucleus (MIN) is similarly affected in Siamese cats but is not shown here.

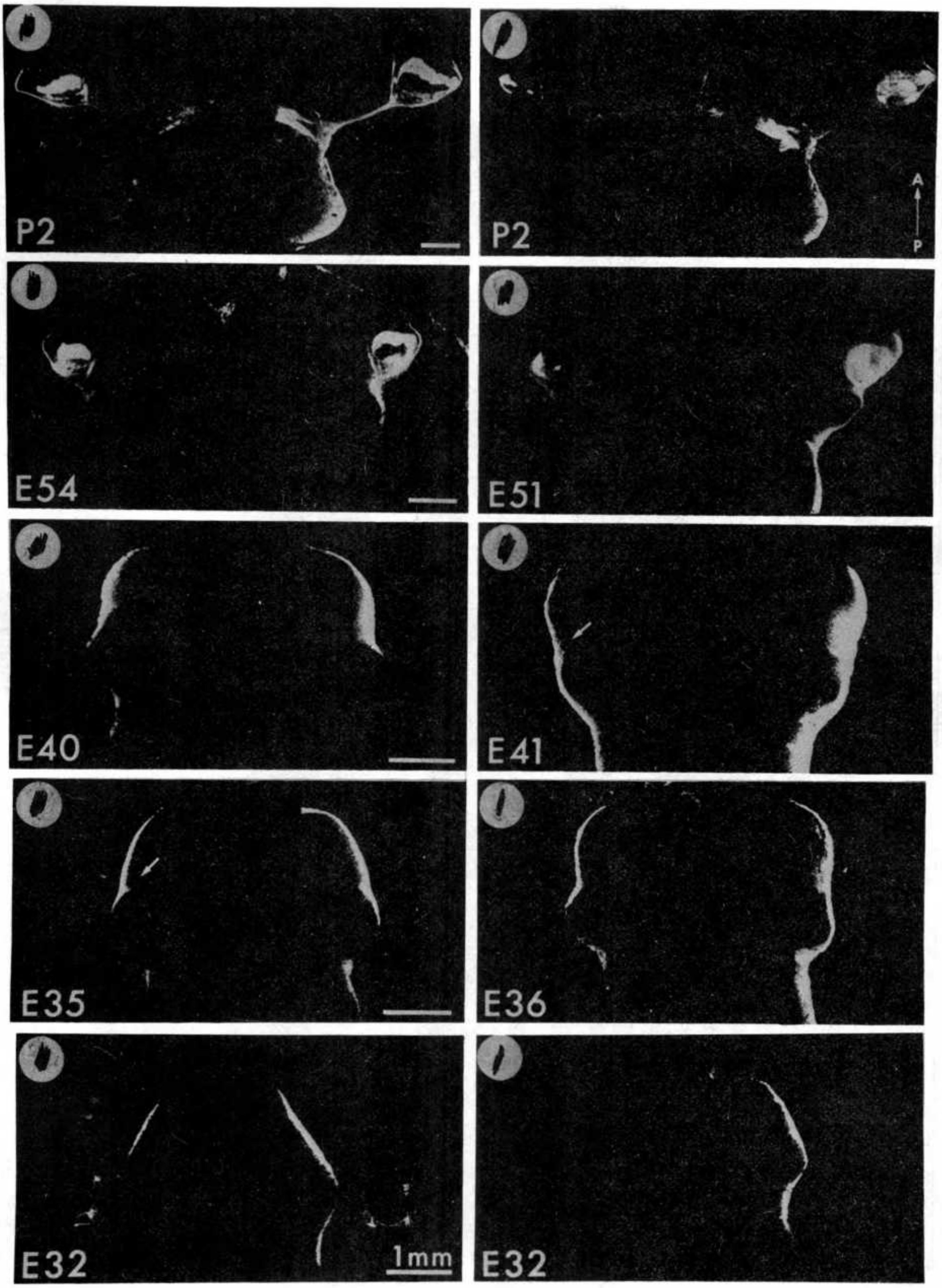

Fig. 2 Two series of darkfield photographs of HRP- or radioactively-labelled retinal afferents in horizontal sections through the lateral geniculate nuclei of normal and Siamese cats to show the progressive development of the retinogeniculate pathway. Postnatal (P) and embryonic (E) ages are indicated, as are the sides contralateral (contra.) and ipsilateral (ipsi.) to the injected eye. Due to the cat's extended gestation period (65 days), reasonable comparisons can be made between normal and Siamese fetuses differing by up to a few days in embryonic age. For each Siamese case, the section shown contained the largest amount of ipsilateral label seen. Label in the top half of each photo is contained within the LGNs and optic tracts. Label in the bottom half lies within the pretectum and superior colliculus. Arrows in *d* and *h* indicate label within the most posterior pole of the LGN. In *f*: A represents anterior, P posterior. The calibration bar (1 mm) for each pair of ages is shown in the left-hand (normal) series. See text for further details.

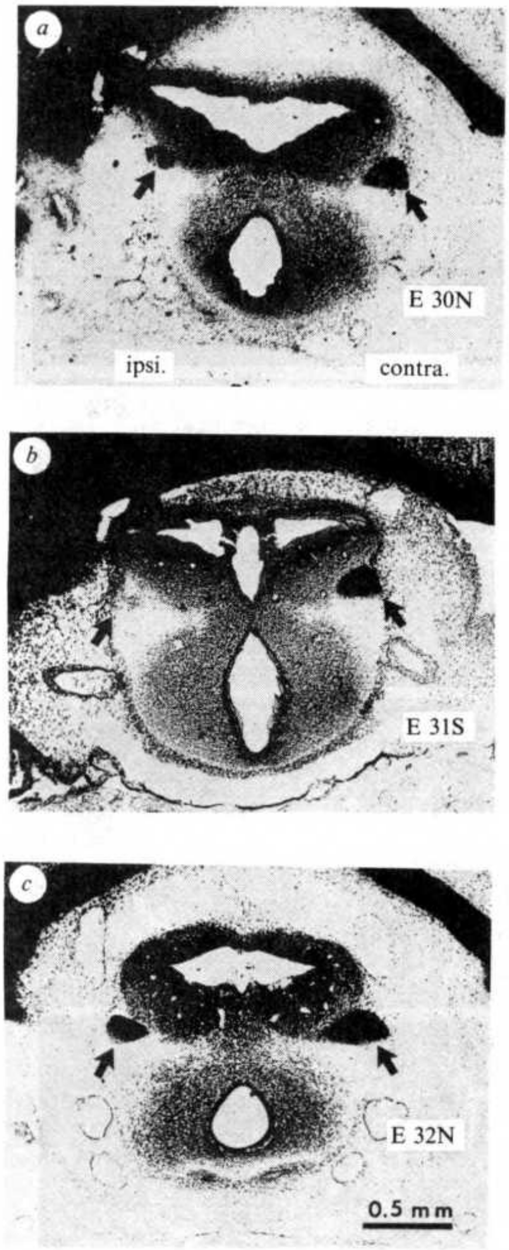

Fig. 3 Brightfield autoradiographs of horizontal sections through the optic tracts immediately adjacent to the chiasm to compare the size of the ipsilateral (ipsi.) and contralateral (contra.) retinofugal projection in three fetuses that received intra-ocular injections of ^{3}H-amino acids. Arrows indicate label (black) in the optic tracts. *a*, Normal E30 fetus; *b*, Siamese E31 fetus; *c*, Normal E32 fetus. The sections were counterstained with cresyl violet. Anterior is up, posterior down. Calibration bar (0.5 mm) is for *a*–*c*.

LGN of Siamese cats. It might arise either as a direct consequence of axonal misrouting at the optic chiasm, as originally proposed by Guillery[3,4], or axons might be routed correctly at the chiasm but subsequently fail to invade the ipsilateral LGN on arrival. We therefore examined the pattern of labelling of ipsilateral and contralateral optic tracts immediately adjacent to the optic chiasm at times when most afferents have not yet grown into the LGN. Figure 3 gives examples of brightfield autoradiographs from fetuses aged between E30 and E32. Substantial label in normal animals is present within the ipsilateral optic tract by E30 (Fig. 3*a*, arrows), roughly 5 days before the time when ipsilateral afferents unambiguously invade the LGN; label has further increased by E32 (Fig. 3*c*). In contrast, at E31 in Siamese fetuses (Fig. 3*b*), the ipsilateral optic tract is only faintly labelled despite extensive label in the contralateral tract. Thus, in Siamese cats the ipsilateral projec-

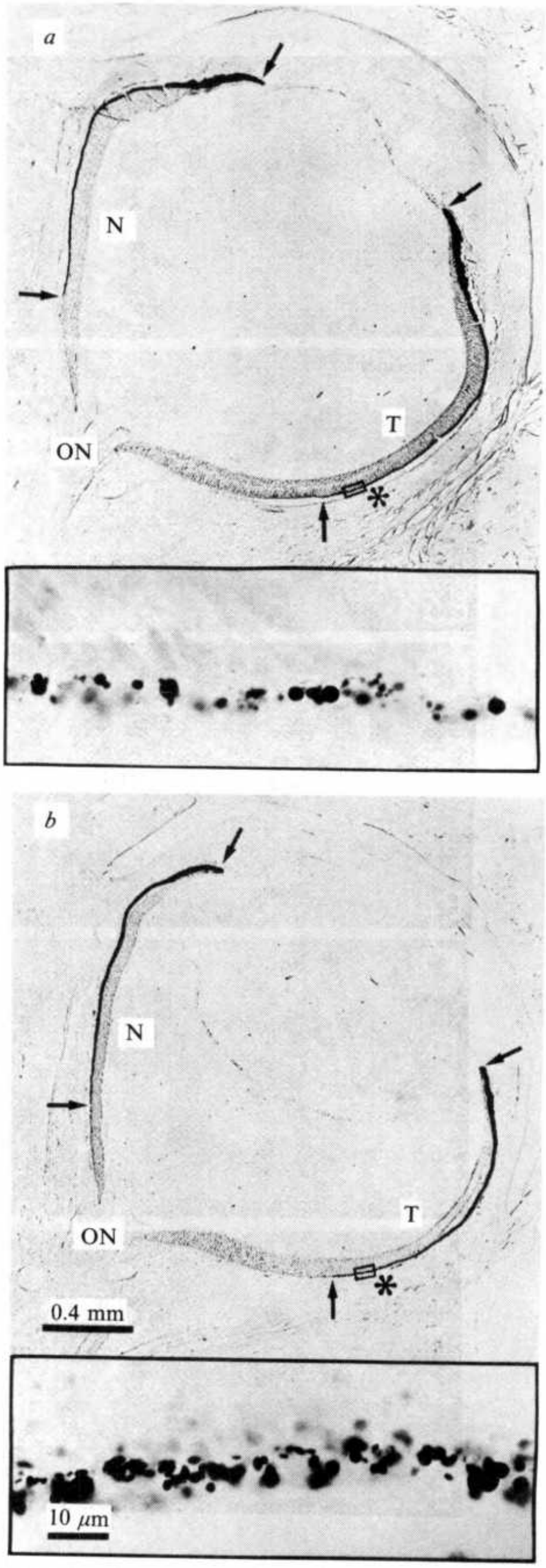

Fig. 4 Brightfield photographs of horizontal sections through the eyes of an E32 Siamese fetus (*a*) and an E32 normal fetus (*b*) to compare the distribution of pigment within the retinal pigment epithelial cells. The sections were counterstained with cresyl violet. Arrows indicate the extent of densely pigmented (black) regions of the epithelium in nasal (N) and temporal (T) retina. ON, optic nerve. Areas enclosed in small rectangles (marked by asterisks) in *a* and *b* are shown at high magnification in the insets beneath to illustrate individual pigment granules that are confined within the pigment epithelial cells. (At these ages, the choroid is unpigmented.)

tion is reduced within the optic tract even before the first retinal afferents reach the vicinity of the developing LGN, thereby supporting the hypothesis that the abnormal projection arises as a consequence of misrouting of growing fibres at the optic chiasm.

It is unknown how the Siamese mutation gives rise to the chiasmatic misrouting of retinal ganglion cell axons seen here. However, it is now well established that an abnormal retinofugal projection is frequently associated with a reduction in adult levels of melanin within the pigment epithelium of the retina. This reduction can be produced by various different mamalian genes, including the Siamese allele[18–20]. In view of this correlation, it has been suggested that melanin itself or another related gene product of the pigment epithelial cell can influence the chiasmatic fate of retinal ganglion cell axons during fetal life[21–23]. We therefore examined the pattern of pigmentation within the retinal pigment epithelium. Surprisingly, we found that the spatial distribution of pigment in our E32 Siamese cats was similar to that in normal cats of the same age (Fig. 4*a*, *b*; compare arrows). However, as can be seen from a comparison of the insets of Fig. 4*a*, *b*, the amount of pigment was somewhat reduced in the Siamese fetus. If pigment does have a role within the retina, then these results suggest that it may do so via an actual reduction in pigment levels. Alternatively, a change in the pigmentation of the developing optic stalk, which in other mammals is known to become transiently pigmented during early embryonic development[21–23], could be the influential factor.

If, as seems likely, optic axons are indeed misdirected at the chiasm in Siamese cats, then it is reasonable to imagine that during normal development, most retinal ganglion cell axons are initially directed to the appropriate side of the brain. It should be emphasized here, however, that our evidence for this conclusion is indirect, as it is based on an examination of the axonal projection pattern. If the present suggestions are substantiated, then the retinogeniculate pathway would represent another example of a system in which directed growth of axons during embryogenesis gives rise to a pattern of connectivity that is retained in the adult. Such directed growth is also found in the development of connections between spinal cord motoneurones and muscles in the chick: motoneurone axons grow directly to the appropriate peripheral muscle masses[24]. In contrast, in the development of the mammalian neocortex, many cerebral cortical neurones initially elaborate axonal connections with both hemispheres and subsequently retract one but retain the other to become either callosal or association projection neurones in the adult[25,26]. Thus, it would seem that the nervous system uses very different strategies in the formation of different sets of connections.

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Adult thymectomy does not alter the proportion of T cells of the Ly 123 subclass

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The murine T cell antigens Ly 1, 2 and 3 have been useful in defining functionally distinct T cell subpopulations[1]. However, the relationship between Ly 123^+ cells and Ly 1^+23^- cells is not clear[2–4]. It has been claimed[5,6] that all cells leaving the thymus are Ly 123^+, the implication being that these must be the precursors of Ly 1^+23^- cells and that maturation involves the loss of Ly 2 post-thymically. Previous work has shown a 50% drop in the proportion of T cells of the Ly 123^+ subclass following adult thymectomy[5]. The interpretation was that the Ly 123^+ cells differentiated into Ly 1^+23^- cells and that no Ly 123^+ migrants from the thymus were replacing them. However, when we directly measured the Ly phenotype of migrant cells within a few hours of their exit from the thymus[7], we found they were mature in phenotype and already contained Ly 1^+23^- cells in the same proportion (~60%)[8] as peripheral T cells. This result suggests that adult thymectomy should not affect the subclass distribution of T cells. We have therefore repeated the adult thymectomy experiments using more precise techniques (flow cytometry and monoclonal antibodies) and the results reported here, in contrast to those of the earlier study[5], did not show any change in the proportion of Ly 123^+ cells after adult thymectomy, even though total T cell numbers were substantially reduced.

In the original studies[5] T cells were considered to consist of Ly 123^+, Ly 1^-23^+ and Ly 1^+23^- subclasses, and after adult thymectomy, a 50% drop in Ly 123^+ cells and an increase in Ly 1^-23^+ and Ly 1^+23^- cells was seen. However, it is now clear that all or most T cells express Ly 1, and the major distinction is between Ly 2^+ (that is, Ly 123 and the so called Ly 23) and Ly 2^- (that is, Ly 1) subclasses[8]. Viewed in this light their data showed a 30% drop in Ly 2^+ cells. For this reason our experiments distinguish T cells primarily on the basis of the presence or absence of Ly 2. With a sensitivity in our assay system of a few per cent, a drop of 30% should be easily detected.

Groups of 4-week-old CBA mice were thymectomized and killed 1, 2 or 4 weeks later. Cells from their mesenteric lymph

Table 1 Proportions of Thy 1^+ and Ly 2^+ cells in spleens and lymph nodes of CBA mice at various times after adult thymectomy

		Thy 1^+ as % of viable nucleated cells			Ly 2^+ as % of Thy 1^+ cells		
		8	14	28	8	14	28
Spleen	Control	27	35	36	43	45	39
	TX	20	28	23	40	44	37
Lymph node	Control	70	77	79	38	34	34
	TX	66	70	74	33	34	39

Mice were thymectomized (TX) at 4 weeks of age and assayed 8, 14 or 28 days later. Controls were untreated, aged matched animals. Percentages are based on a sample of 25,000 cells taken from a pool of three animals.

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Table 2 Effect of adult thymectomy on T cells in C57BL/6 mice

Organ	Group	No. of animals	% Thy 1^+ (of total cells)	Total Thy 1^+ (% of control)	% Ly 2^+ (of total) T cells)
Spleen	Control	4	34	100	44
	Sham TX	4	29	95	43
	TX (*a*)	3	22	59	44
	TX (*b*)	3	18	60	47
Lymph node	Control	4	73	100	46
	Sham TX	4	62	92	44
	TX (*a*)	3	66	38	49
	TX (*b*)	3	69	49	48

Mice were thymectomized (TX) at 7 weeks of age and assayed 3 weeks later. Controls were untreated age matched animals, while sham TX were age matched animals which were anaesthetized and the chest opened without touching the thymus. Percentages are based on a sample of 50,000 cells taken from a pool of three or four animals. TX (*a*) and TX (*b*) are duplicate groups.

nodes or spleens were stained simultaneously with monoclonal anti-Thy 1 and anti-Ly 2. Age-matched normal control mice were similarly stained. The methods and results obtained on a computer-linked fluorescence-activated cell sorter[9] are shown in Fig. 1. Thy 1^+ and Ly 2^+ cells could be unambiguously identified. The staining pattern was highly reproducible, and despite the apparent complexity of the staining protocol, controls were clear cut. The results of this approach are shown in Table 1. At all the times tested after thymectomy, the proportion of Thy 1^+ cells was reduced (most markedly in spleen as previously noted[10]), but the proportion of Ly 2^+ cells amongst the remaining Thy 1^+ cells was unchanged.

The original observations of Cantor and Boyse[5] were made on slightly older (7 week) C57BL/6 mice, so we repeated the experiment with 7-week-old C57BL/6 and CBA mice and, as they did, examined them 3 weeks later. Tables 2 and 3 show the results of these experiments. Three weeks after thymectomy, there was a slight drop in the proportion of T cells amongst the lymphocyte population as a whole and a substantial drop of around 50% in the absolute T cell numbers. Despite these changes the proportion of Ly 2^+ cells amongst the Thy 1^+ cells remained constant.

Using a similar staining protocol for Ly 1 and Thy 1, we found there was also no change in the small Thy 1^-, Ly 1^+ population 3 weeks after thymectomy (data not shown). This Thy 1^-, Ly 1^+ population makes up a few per cent of the cells in normal spleen or lymph node (ref. 8 and R.S., unpublished results). They would fall in the Thy 1^-, Ig^- group in the experiments shown in Fig. 1, but would probably have been included in the Ly 1^+ group in the experiments of Cantor and Boyse. They do not account for the differences in our results. It is interesting to note that a comparison of the % Thy 1^+ with the total Thy 1^+ (Tables 2, 3) indicates that adult thymectomy must also have caused a substantial drop in non-T (mostly B) cell numbers, indicating that even the adult B cell pool remains to some extent dependent on an intact thymus.

Table 3 Effect of adult thymectomy on T cells in CBA mice

Organ	Group	No. of animals	% Thy 1^+ (of total cells)	Total Thy 1^+ (% of control)	% Ly 2^+ (of total T cells)
Spleen	Control	3	29	100	44
	Sham TX	3	31	73	40
	TX (*a*)	3	25	48	42
	TX (*b*)	3	23	72	41
Lymph node	Control	3	66	100	36
	Sham TX	3	63	117	35
	TX (*a*)	3	68	46	37
	TX (*b*)	3	73	25	35

Mice were thymectomized (TX) at 7 weeks of age and assayed 3 weeks later. Untreated controls and sham TX animals were age matched. Percentages are based on a sample of 50,000 cells taken from a pool of 3 animals. TX (*a*) and TX (*b*) are duplicate groups.

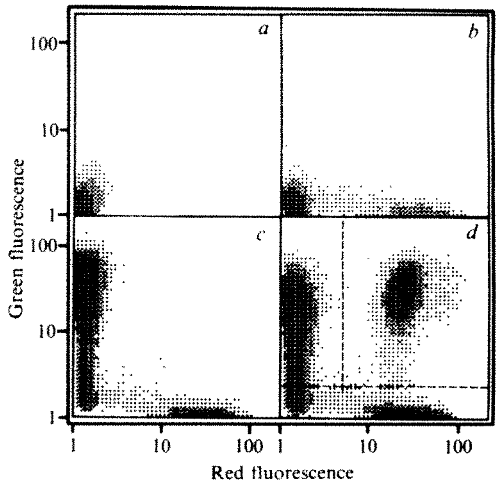

Fig. 1 Typical two-dimensional staining patterns of normal lymph node cells stained simultaneously with anti-Thy 1 and anti-Ly 2 and controls. *a*, Unstained cells; *b*, cells stained with rhodamine-conjugated rabbit anti-mouse immunoglobulin (Rh.RαMIg). B cells can be clearly distinguished from immunoglobulin-negative cells; *c*, cells stained with Rh.RαMIg, followed by rat immunoglobulin to saturate free binding sites on the Rh.RαMIg, followed by fluoresceinated rat monoclonal anti-Thy 1.2 (30-H12, ref. 19). T cells and B cells can be clearly distinguished and a small proportion of double negative cells (null cells) remains; *d*, cells stained with a rat monoclonal anti-Ly 2 (53-6.7, ref. 19) followed by the same staining as *c*. The Rh.RαMIg, which recognizes both mouse immunoglobulin and rat immunoglobulin, now stains both B cells and Lyt 2^+ cells, the latter being distinguished from B cells by the presence of Thy 1. The dotted lines show the levels used to define Thy 1^+ and Ly 2^+ cells and the major subpopulations (Thy 1^+ Ly 2^+; Thy 1^+ Ly 2^-; Thy^-) are easily distinguished. Among the Ly 2^- cells, there is no distinct separation between Thy 1^+ and Thy 1^- (*d*). Thy 1^- cells were arbitrarily chosen as those with levels undistinguishable from the negative controls (*a* and *b*). The data in each box represent 50,000 cells, plotted as a contour diagram. This form of plotting gives little information on peak heights, but clearly shows degrees of separation. The single peak in *a* contains 50,000 cells, while the other peaks vary (for example, the Lyt 2^+, Thy 1^+ peak in *d* contains about 17,000 cells). 3×10^6 cells were stained in each case and suspensions were washed between stages. Cells were analysed by flow cytometry (FACS II Becton-Dickenson) using a logarithmically amplified, two colour, single laser system linked to a PDP-11/34 computer. The computer produced the figures shown above and calculated the proportion of cells in each of the categories defined by the dotted lines in *d*. All the numbers in the tables were obtained from this kind of analysis. The slight increase in apparent red staining with increasing Thy 1 levels on red negative cells (*c* and *d*) is an artefact of the electronic signal separation system which occurs when working with low level signals in logarithmic mode.

In view of the marked discrepancies with the earlier data two technical questions immediately arise. How do the two techniques for measuring Ly phenotypes differ and was thymectomy in our experiments complete? In the flow cytometric analysis described here monoclonal reagents were used, 50,000 cells were analysed per sample, and background staining levels (that is, rhodamine positive, Thy 1^+ cells in preparations stained only with anti-Thy 1 and anti-immunoglobulin antibodies, Fig. 1*c*) were in the range of 1–2%. Thus, the actual numbers of Ly 2^+ cells counted in various samples ranged from about 17,000 in control lymph node to 5,000 in thymectomized spleens. In the sequential ^{51}Cr-release cytotoxic analysis, populations of 20–50% were estimated by subtraction following two sequential killing steps each with a background of up to 12%. Furthermore, as different subpopulations may take up different amounts of ^{51}Cr, the proportion of ^{51}Cr released may not correspond to the proportion of cells killed. Finally there is the complexity of complement-mediated cytotoxicity, as resistance to lysis can result from factors other than density of surface antigen[8]. For these reasons we believe the flow cytometric analysis to be the more reliable method. With regard to completeness of thymectomy, each animal in our experiments was examined macroscopically and histologically and no evidence of residual thymus was found. Furthermore, the total number of T cells was reduced by about 50% following the operation, suggesting that thymectomy was complete.

In conclusion, the data reported here are in agreement with the predictions which come from the direct measurement of the Ly distribution[3,7] and phenotypic maturity[11,12] of thymus migrants in adult mice. They are also in agreement with other recent reassessments of the Cantor and Boyse model. These showed (1) that there is a significant population of Ly 1 cells in the thymus[2] and (2) that T cells in newborn mice (which are presumed to be mostly recent thymus emigrants) are similar in Ly phenotype to peripheral T cells in adults[12,14]. Taken together, these studies support the hypothesis that thymus migrants are mature and that the division between Ly 123$^+$ and Ly 1$^+$23$^-$ cells must occur before emigration from the thymus. In fact, if data showing that Ly 1$^+$ cells appear earlier in ontogeny than Ly 123$^+$ cells are considered[15-17], it is possible to suggest that the division occurs at the earliest stem cell stage[2,3,16].

Finally, although these data support the hypothesis that thymus migrants in adults are phenotypically mature, it is possible that in some circumstances (for example, at specific stages during ontogeny) the thymus does give rise to migrant cells of 'immature' phenotype, a point which is under investigation[3,13,18] but not yet resolved. In addition, it should be made clear that these experiments and our other published data concerning thymus migrants[3,7,11,12] do not necessarily exclude a cortical origin for some or all thymus migrants. Nor do they clarify the relationships of the cortex and medulla. They do however rule out the emigration of immature cells directly from the cortex of adult mice and necessitate a re-analysis of the concept of post-thymic maturation.

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Absence of myosin-like immunoreactivity in stereocilia of cochlear hair cells

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Movements of the stiff sensory hairs, the stereocilia, which extend outwards from the apical surface of the hair cells in the auditory organ, are thought to have a key role in the process of transduction of sound energy into electrical impulses. Each stereocilium is supported by a paracrystalline axial bundle of actin filaments, which have identical polarities and are extensively cross-linked[1-4]. Recent immunohistochemical observations on whole mounts of the guinea pig organ of Corti indicated myosin-like immunoreactivity in association with hair cell stereocilia[5]. Considering the steric situation, it is, however, difficult to imagine any functional interaction between the bundled actin filaments and the assumed myosin molecules. Here we report that in semi-thin, transverse sections of quick-frozen, freeze-dried and plastic-embedded guinea pig organ of Corti, myosin-like immunostaining was restricted to the apical cytoplasm of hair cells and was not detected along the stereocilia. With respect to the immunohistochemical distribution of actin, myosin and the muscular Z-line protein, α-actinin, the apex of hair cells strongly resembles the intestinal epithelial brush border[6,7].

Specific immunostaining of cochlear hair cells was achieved using antibodies directed against purified non-muscle myosin from calf thymus (Fig. 1*a*, *b*); these antibodies have previously been shown to react with a broad spectrum of non-muscle cells[8-10]. Immunostaining was completely abolished when the antibodies were absorbed with an excess of thymus myosin. The bulk of myosin-specific immunofluorescence was confined to the apex of the hair cells, the so-called cuticular plate (Fig. 2*a*). A narrow, rather faint, fluorescent band extended from the cuticular plate downwards along the lateral cell border of the outer but not the inner hair cells. Antibodies to striated muscle (chicken pectoral) and smooth muscle (human uterus, chicken gizzard)[11] myosin did not react with either the hair cells and their stereocilia or any other cell type of the organ of Corti,

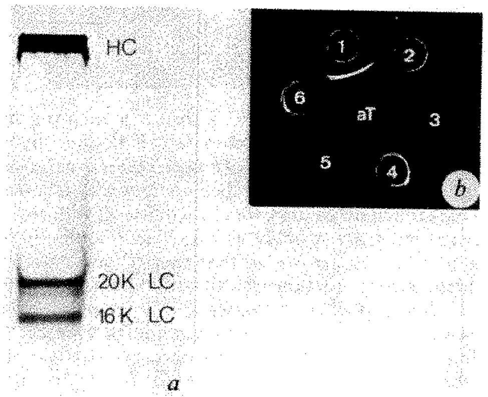

Fig. 1 *a*, 0.1% SDS-(10%) polyacrylamide gel electrophoresis of calf thymus myosin which was further purified by DEAE-cellulose ion-exchange chromatography and used to prepare antibodies. HC, myosin heavy chain; 20K LC, 20,000-M_r myosin light chain; 16K LC, 16,000-M_r myosin light chain. *b*, Double immunodiffusion of anti-calf thymus myosin (aT, centre well) against thymus myosin (1), chicken gizzard (2) and pectoral myosin (3); human uterine (4) and placental (5) myosin, and porcine arterial myosin (6).

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indicating that these cells contain an immunologically distinct type of myosin. This finding is in contrast to the observations of Macartney *et al.*[5], who described staining of cochlear stereocilia with antibody to human uterus myosin. Although we cannot offer a definite explanation for this discrepancy, there are two possibilities to consider. (1) The myosin preparation used by Macartney *et al.*[5,12] contained minor amounts of some other proteins running in SDS-polyacrylamide gel electrophoresis between the heavy and presumed light chains of myosin (Macartney *et al.*[12] discussed the possibility of contamination of their antigen with actin or tropomyosin) and it is possible that the antibody to uterus myosin contained additional immunoglobulins directed against these unknown minor protein components. (2) Macartney *et al.*[5] used whole mounts of the organ of Corti for immunohistochemical staining. This method of tissue handling, however, is sensitive to a particular optical artefact known as 'light piping' of stereocilia that might give the impression of specific immunofluorescence (for detailed discussion, see ref. 13). In the present study we applied immunostaining to 0.5-μm-thick sections of quick-frozen, freeze-dried, plastic-embedded tissue. This method avoids diffusion artefacts and allows examination at high resolution. Considerable light piping will probably not occur in these conditions because the thickness of the sections is within the range of the thickness of an individual stereocilium (0.4–0.8 μm)[2,3].

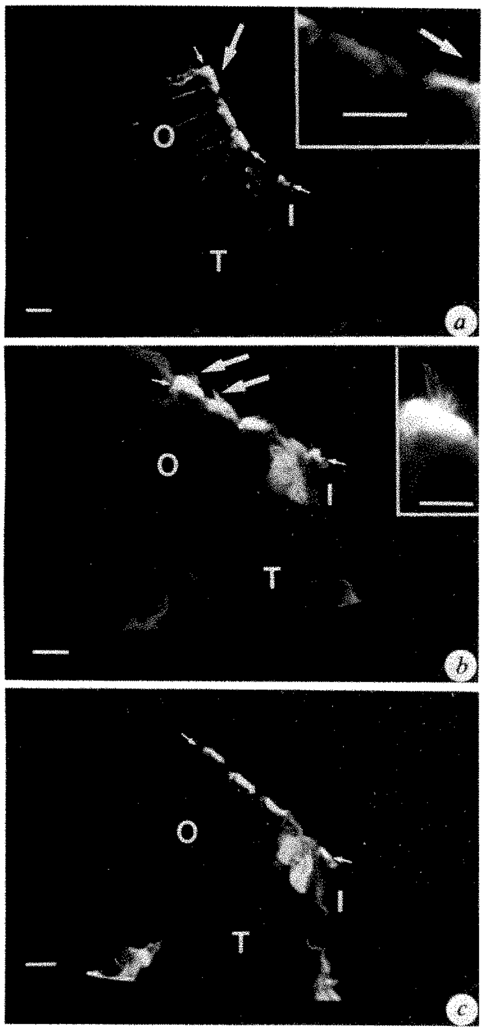

Fig. 2 *a–c*, Semi-thin section (0.5 μm) of guinea pig organ of Corti stained with rabbit antibodies to calf thymus myosin (*a*), chicken gizzard actin (*b*) and chicken skeletal muscle α-actinin[10] (*c*). Large arrows in *a* and *b* indicate stereocilia (shown in the insets at higher magnification), which do not react with anti-myosin antibody (*a*) but react strongly with anti-actin antibody (*b*). The cuticular plate (small arrows) of the inner (I) and outer (O) hair cells display a strong reaction with all antibodies shown. The inner tunnel (T) is bounded by the outer and inner pillar cells which exhibit a strong fluorescence with antibodies to actin (*b*) and α-actinin (*c*). Scale bar, 10 μm. The bony cochlea was removed under ether anaesthesia and was rapidly frozen by placing in melting isopentane cooled with liquid nitrogen. Freeze-drying was performed at 10^{-4} torr starting with a temperature of -100 °C, which was increased stepwise to room temperature over 3 days. Freeze-dried bony cochleae were vacuum-embedded in Epon. Semi-thin sections (cut with glass knives on a Reichert Ultracut microtome) were mounted on glass slides and the resin was removed as described elsewhere[18]. The antibody concentrations used were 0.5 mg ml^{-1} for the primary antibodies and 0.1 mg ml^{-1} for the FITC (fluorescein isothiocyanate)-labelled goat anti-rabbit IgG (Miles). Dilution of antibodies and rinsing steps were done in phosphate-buffered saline, *p*H 7.2 at room temperature.

As expected, antibodies to actin from chicken gizzard reacted strongly with both the cuticular plate and the stereocilia of the hair cells (Fig. 2*b*). There was a strong actin-like staining in the cytoplasm of the outer and inner pillar cells and the apical processes of phalangeal cells (both structures did not react with anti-myosin antibody). Antibodies to α-actinin from chicken pectoral muscle[10] produced very intense staining in the cuticular plate of outer and inner hair cells but not in their stereocilia (Fig. 2*c*). As with anti-actin, anti-α-actinin antibody displayed a high affinity for the apical processes of phalangeal cells and for the outer and inner pillars, indicating that both actin and α-actinin are major constituents of the prominent cytoskeletal apparatus present in these supporting cells. In the pillars this apparatus consists of bundles of microtubules intermingled with microfilaments (actin ?) and electron-dense reticular material (α-actinin ?)[14]. The apex of phalangeal processes is filled with electron-dense reticular material and microfilaments[14].

Thus, the present findings provide evidence that actin, myosin and α-actinin are concentrated within the apex of cochlear hair cells. While actin is present both the stereocilia and the cuticular plate, myosin and α-actinin are obviously absent from the stereocilia and are restricted to the cuticular plate. A similar distribution of these contractile proteins has been demonstrated in the intestinal brush border[6,7,15]. Stereocilia have been shown to react like intestinal microvilli with antibodies to fimbrin (an actin cross-linking protein)[4] but in the present work and in a previously published study[4], they apparently did not react with antibodies to villin[16] (a 95,000-molecular weight (M_r) protein that bundles and severs actin filaments in a Ca^{2+}-dependent manner[17]). This observation indicates differences in the molecular composition of the actin filament bundle present in stereocilia and microvilli. Motile events have so far not been reported in hair cells and their stereocilia. Therefore, it remains to be shown whether myosin and α-actinin serve merely to cross-link and tighten the web of actin filaments within the cuticular plate or whether these proteins constitute a contractile apparatus that might actively modify mechanical properties of the apical portion of cochlear hair cells.

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Meta-vinculin—a vinculin-related protein with solubility properties of a membrane protein

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The localization of vinculin, an actin-binding protein[1,2] of molecular weight 130,000 (130K) at a variety of cytoskeletal-associated membrane specializations including the zonula adherens of intestinal epithelial cells[3], fascia adherens of intercalated discs[3], focal adhesion plaques in fibroblasts[4,5], and costameres (sarcolemmal domains in periodic register with the I-bands of subjacent myofibrils) in cardiac and skeletal muscle[6], suggests that vinculin could link actin to cell membranes. This hypothesis is supported by immuno-ultrastructural studies which show that vinculin is closely apposed to the plasma membrane at sites where actin-containing microfilaments terminate[3,7]. But since vinculin has the solubility properties of a peripheral membrane protein[4], there would also need to be an integral membrane component to which it bound to link actin filaments to the plasma membrane. Our efforts to identify the presumed membrane binding site for vinculin resulted instead in the detection of a 150K protein which we term meta-vinculin (Greek *meta* among, with; in the sense of closely related to), that is immunologically related to 130K vinculin. Unlike vinculin, meta-vinculin has the solubility properties of an integral membrane protein. Given its location within the cell, it is possible that meta-vinculin is an integral membrane anchor protein for actin filaments.

Unfixed cryostat sections of chicken gizzard were extracted with buffers designed to solubilize either peripheral or integral membrane proteins[8,9] in an attempt to remove vinculin from its location in dense plaques on the smooth muscle plasma membrane (Fig. 1*a*)[3]. The effect of the extractions was assessed by subsequent indirect immunofluorescence staining of the sections with affinity purified anti-vinculin. Because vinculin is isolated by low ionic strength extraction of minced gizzard[4], for example, with 2 mM Tris/1 mM EGTA, *p*H 9.0[10] or 1 mM $KHCO_3$, *p*H 7.5[11], we expected to observe a dramatic decrease in fluorescence when cryostat sections were extracted in these buffers for various times (up to 24 hours) and then fixed and processed for indirect immunofluorescence. However, the low ionic strength buffers extracted barely 20% of the membrane associated vinculin as evidenced both by slightly dimmer staining (Fig. 1*c*, *e*) and by microspectrophotometry (data not shown). Neither was vinculin extracted to any greater extent by the following buffers known to disrupt electrostatic interactions between proteins: 50 mM Na acetate *p*H 5.4 (low *p*H); 0.01 M NaOH *p*H 11.5 (high *p*H); 1.0 M Tris-HCl *p*H 7.4 (high salt); 0.6 M KI (chaotropic agent); 50 mM Tris plus 0.1–0.6 M KCl *p*H 8 (buffers of various ionic strengths); 2 mM Tris plus 5 mM EGTA plus 5 mM EDTA, *p*H 8.0 (chelators). Many integral membrane proteins can be solubilized by non-ionic detergents which primarily disrupt hydrophobic interactions. However, 1% Triton X-100 in 10 mM NaCl, 1.5 mM $MgCl_2$, 10 mM Tris *p*H 7.4 (1% Triton buffer) extracted only about 20% of the vinculin from unfixed cryostat sections (Fig. 1*g*) even though the plasma membranes were disrupted as evidenced by a phase-clear area outlining each smooth muscle cell. Interestingly, the vinculin that remained behind with the detergent-insoluble residue was still organized as in the control sections; the outline of each cell could be clearly discerned by the fluorescence of the anti-vinculin stain. In contrast to all other treatments, we found that over 90% of the vinculin could be extracted by a combination of 1% Triton X-100 and 0.6 M Tris-HCl *p*H 7.4 (Fig. 1*i*). These results suggest that to

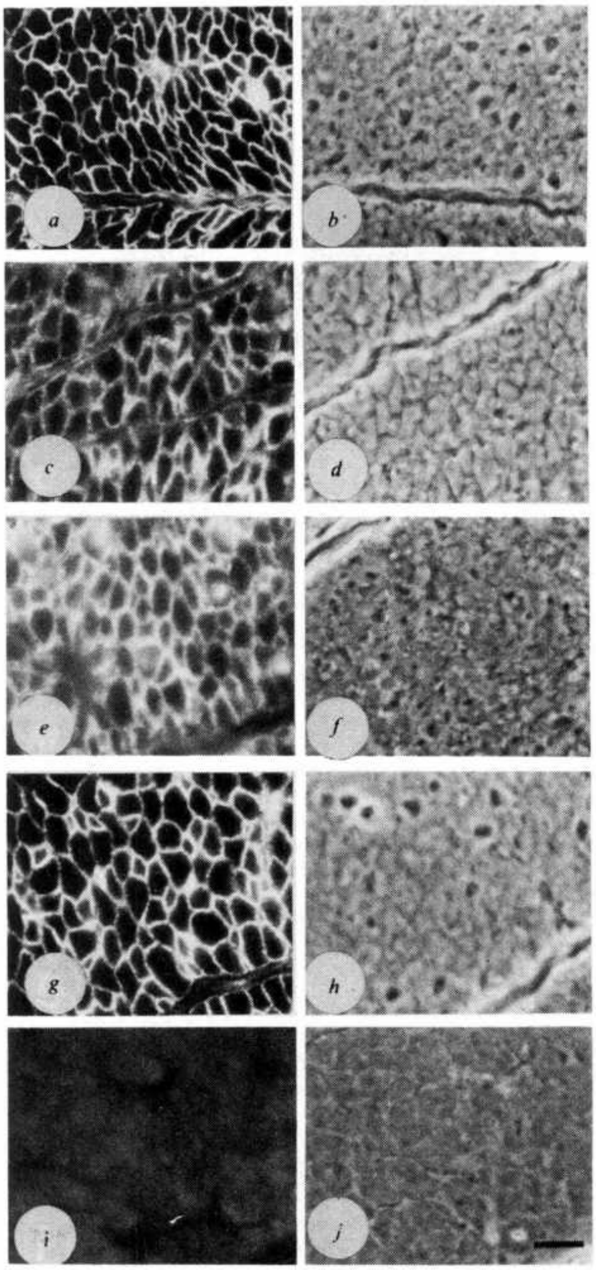

Fig. 1 Conditions for extraction of immunoreactive vinculin from cryostat sections of gizzard. The gizzard from a freshly killed chick was removed, cut quickly into small pieces and dropped into freezing −80 °C isopentane. The frozen tissue was transferred to powdered dry ice and frozen onto a copper chuck with OTC compound. 4 μm sections were cut on a Harris cryostat at −20 °C and mounted immediately on gelatin-coated glass slides. Sections were extracted in the indicated buffer for the following lengths of time: 1% Triton in 10 mM NaCl, 1.5 mM $MgCl_2$, 10 mM Tris *p*H 7.4 or 1% Triton + 0.6 M Tris-HCl *p*H 7.4, 3 min; 1 mM $KHCO_3$ *p*H 7.5 or 2 mm Tris + 1 mM EGTA, *p*H 9.0, 30 min. After extraction, sections were rinsed in Dulbecco's-PBS (D-PBS), fixed for 5 min in 3% paraformaldehyde *p*H 7.3 and then processed for indirect immunofluorescence. After incubation with 120 $\mu g\ ml^{-1}$ affinity purified anti-vinculin for 45 min, sections were washed extensively in D-PBS. Affinity purified fluorescein conjugated goat anti-rabbit Igs (50 $\mu g\ ml^{-1}$) were then added to the sections. After incubation for 30 min, sections were washed in D-PBS and observed using an Ortholux II fluorescence microscope equipped for epifluorescence illumination with Leitz KG1, BG23, K480 filters and H2 cube. Photomicrographs were recorded with a Vario-Orthomat camera on Kodak Tri-X pan black and white film, ASA 1000. *a*,*b*, Unextracted gizzard; *c*,*d*, 1 mM $KHCO_3$ *p*H 7.5 extraction; *e*,*f*, 2 mM Tris/1 mM EGTA *p*H 9.0 extraction; *g*,*h*, 1% Triton *p*H 7.4 extraction; *i*,*j*, 1% Triton + 0.6 M Tris *p*H 7.4. *a*,*c*,*e*,*g*,*i*, Fluorescence micrographs; *b*,*d*,*f*,*h*,*j*, phase micrographs. Scale bar, 10 μm.

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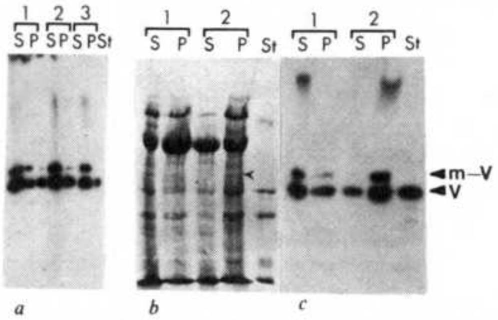

Fig. 2 Detection of 150K meta-vinculin and comparison of its solubility properties with those of 130K vinculin. A 60-mg fragment of gizzard from a freshly killed chick was homogenized in 1.0 ml of the appropriate extraction buffer which included the following protease inhibitors: (final concentration): leupeptin (10 μg ml^{-1}), antipain (20 μg ml^{-1}), Trasylol (500 units ml^{-1}, benzamidine (100 μg ml^{-1}), chymostatin (10 μg ml^{-1}) and pepstatin (10 μg ml^{-1}). The homogenates were spun at 100,000*g* for 60 min in a SW 50.1 rotor at 4 °C. An equal volume of 2 × Laemmli SDS sample buffer was added to aliquots of supernatants. 500 μl of 2× sample buffer was used to solubilize pellets. Equal volumes of supernatants and pellets were electrophoresed on a 5% Laemmli SDS polyacrylamide mini gel. For immunoblots, the proteins were electrophoresed onto nitrocellulose paper[12]. Vinculin and meta-vinculin were detected by incubating the nitrocellulose paper with affinity-purified anti-vinculin (120 μg ml^{-1}). After extensive washing in Nonidet P-40 (NP-40) buffer (0.05 M Tris, 0.15 M NaCl, 0.05% NP-40, 0.02% NaN_3, 0.1% bovine serum albumin *p*H 8.0), the paper was incubated with ^{125}I-Protein A which was diluted in the NP-40 buffer. The washing was repeated, the paper was dried and then exposed to Kodak XR-5 X-ray film with a Cronex lightening-plus intensifying screen. Immunodetection of proteins in the SDS gel itself was done by the method of Adair *et al.*[18]. *a*, Autoradiogram of immunoblot. 1, 1% Triton + 0.6 M Tris-HCl *p*H 7.4 extraction, supernatant (S) and pellet (P); 2, 1 mM $KHCO_3$ *p*H 7.5 extraction; 3, 2 mM Tris/1 mM EGTA *p*H 9.0 extraction; St, Standards: filamin, 250K; vinculin, 130K; alpha-actinin, 100K; and bovine serum albumin, 68K. *b*, Coomassie blue stained gel. 1, 1% Triton + 0.6 M Tris-HCl *p*H 7.4 extraction, supernatant (S) and pellet (P); 2, 1 mM $KHCO_3$ *p*H 7.5 extraction. *c*, Autoradiograph of anti-vinculin/^{125}I-Protein A overlay of the same gel shown in *b*.

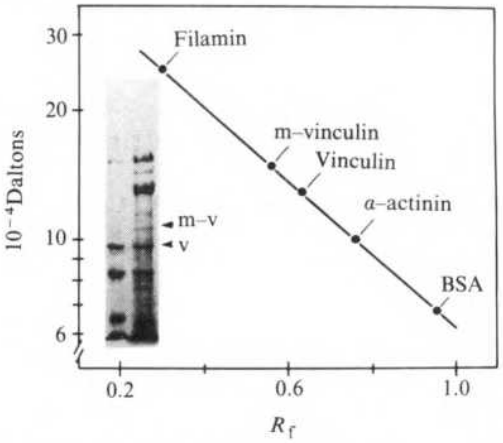

Fig. 3 Estimation of the molecular weight of meta-vinculin by SDS gel electrophoresis. A 100,000*g* supernatant of a 1% Triton + 0.6 M Tris-HCl *p*H 7.4 extract (prepared as described in Fig. 2) was run on a 5% polyacrylamide mini gel. Meta-vinculin and vinculin were localized in the gel by the antibody overlay technique described by Adair *et al.*[18]. Lane 1, standards: Filamin, 250K; vinculin, 130K; α-actinin, 100K; bovine serum albumin, 68K. Lane 2, supernatant of 1% Triton + 0.6 M Tris-HCl *p*H 7.4 extract. Vinculin and meta-vinculin are marked by arrowheads.

solubilize the bulk of the vinculin it is necessary to disrupt two types of linkages—one electrostatic and the other hydrophobic.

Although these data show that vinculin can be extracted efficiently only in the presence of high salt plus detergent, vinculin is conventionally isolated from a low ionic strength extraction of fresh or frozen chicken gizzard[4,10,11]. To assess the basis for this apparent discrepancy we extracted fresh gizzards with various buffers in the presence of protease inhibitors and analysed the 100,000*g* supernatants and pellets by SDS gel electrophoresis followed by an immunoblot[12] developed with affinity purified anti-vinculin. The results (Fig. 2) not only confirm the immunofluorescence observations but also reveal that affinity purified anti-vinculin recognizes two molecules: vinculin and a protein (meta-vinculin) with a slightly higher molecular weight than vinculin. The combination of 1% Triton and 0.6 M Tris-HCl *p*H 7.4 solubilizes most of the vinculin and more than 90% of the meta-vinculin (Fig. 2*a*-1), whereas the low ionic strength buffers (1 mM $KHCO_3$ *p*H 7.5 or 2 mM Tris/1 mM EGTA *p*H 9.0) extract much less of the vinculin and less than 10% of the meta-vinculin (Fig. 2*a*-2 and 2*a*-3). Figure 2*b*, *c* shows that the meta-vinculin detected by an autoradiograph co-aligns with a distinct Coomassie blue stained band (Fig. 2*b*, arrow) which has the appropriate solubility properties of meta-vinculin. In this experiment (Fig. 2*c*), as well as in several but not all other experiments, we also detected a very high molecular weight (>250,000) protein that also reacts with anti-vinculin. Because its presence is variable and unpredictable, the nature of this very high molecular weight cross-reactive material remains unclear. The apparent molecular weight of meta-vinculin was estimated on SDS gels under reducing conditions and is approximately 150,000 (Fig. 3).

To rule out the possibility that detection of the 150K protein was due to the presence of contaminating antibodies, we affinity purified vinculin antibody from immune serum in the following manner[13]: vinculin was electrophoresed on a 5% preparative SDS gel which gave good separation of vinculin from meta-vinculin. The 130K band was excised from the gel, and electrophoresed onto nitrocellulose paper, which was then used to affinity purify anti-130K vinculin. An immunoblot of the 100,000*g* supernatant of a 1% Triton plus 0.6 M Tris extract of fresh gizzard proves that anti-130K vinculin also reacts with 150K meta-vinculin (Fig. 4).

The basis for the antigenic relationship between vinculin and meta-vinculin is unknown. It is possible that 130K vinculin isolated by published procedures is a proteolytic fragment of the 150K meta-vinculin. Alternatively, meta-vinculin and vinculin may be products of two different genes which share some degree of homology, or of a single gene whose transcript is spliced differentially to yield molecules with common and unique functional properties as is the case for secreted and membrane-bound IgM[14].

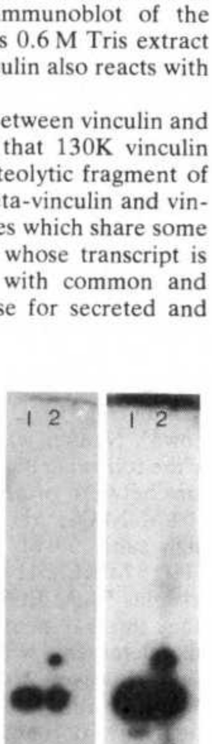

Fig. 4 Anti-vinculin affinity-purified on 130K vinculin also recognizes 150K meta-vinculin. Vinculin (0.5 mg) isolated by low ionic strength extraction of frozen chicken gizzards was electrophoresed on a preparative 5% SDS polyacrylamide gel. After straining the gel for 5 min, the 130K vinculin band was cut out. Vinculin was then electrophoretically transferred to nitrocellulose paper. The paper was incubated with anti-vinculin serum overnight and then washed. Antibody which bound to the 130K protein was eluted with 0.05 M acetic acid, neutralized immediately, and then dialysed overnight against phosphate-buffered saline (PBS) *p*H 7.4. This affinity-purified antibody was then tested for its ability to identify meta-vinculin and vinculin in an immunoblot of the 100,000*g* supernatant of a 1% Triton + 0.6 M Tris-HCl *p*H 7.4 extract of fresh chick gizzard prepared as described in Fig. 2. Lane 1, vinculin standard; lane 2, supernatant of a 1% Triton + 0.6 M Tris-HCl *p*H 7.4 extract. Exposure times were 12 h and 72 h.

Feramisco *et al.*[15] described a 152K protein which is restricted to smooth muscle, and is immunologically related to vinculin, but does not appear to be a proteolytic fragment of vinculin. Possibly this 152K protein is the protein meta-vinculin which we have defined on the basis of solubility properties as well as shared antigenicity with vinculin; however, we do not yet have sufficient information to make a comparison. It will also be of interest to determine if there is a relationship between meta-vinculin and chicken gizzard caldesmon[16], a 150K protein which binds F-actin and is regulated by calcium and calmodulin.

Unlike vinculin, meta-vinculin has the solubility properties of an integral membrane protein. Although it is possible that the low solubility of meta-vinculin in any of the tested solvents other than 1% Triton plus 0.6 M Tris simply reflects tight binding of a peripheral protein to an integral membrane protein, it seems more likely that it reflects differences in the type of bond formed with the plasma membrane. Membrane-associated proteins are divided into peripheral or integral protein classes depending on their solubility properties. Those proteins requiring detergent for solubilization (integral membrane proteins) have been found, with few exceptions, to contain a hydrophobic domain which is buried in the lipid bilayer[17]. Based on these considerations, we speculate that meta-vinculin might be an integral membrane protein and as such, a strong contender for the role of directly linking actin to the plasma membrane.

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Ca^{2+}-free perfusion of rat heart reveals a $(Na^+ + K^+)$ATPase form highly sensitive to ouabain

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The sensitivities of the cardiac $(Na^+ + K^+)$ATPases to inhibition by ouabain have been shown to correlate with the pharmacologically active digitalis concentrations in the various source species[1]. The rat heart enzyme is generally considered to be insensitive to ouabain with half-maximal inhibition at $\sim 6 \times 10^{-5}$ M ouabain[2,3] and a K_D for binding[3–7] of $1–6 \times 10^{-5}$ M. However, recent data[5–7] showed that rat cardiac sarcolemma preparations can bind ouabain with high affinity (k_D $1–3 \times 10^{-7}$ M) with no detectable concomitant enzyme inhibition *in vitro* but producing a sustained positive inotropic effect *in vivo*. In the present study, we measured the $(Na^+ + K^+)$ATPase inhibition by ouabain in sarcolemma-enriched fractions obtained from rat hearts relaxed by perfusion with Ca^{2+}-free solutions. In these conditions, two enzyme forms having high and low sensitivities to ouabain were found (half-maximal inhibitions with 1.2×10^{-8} and 6×10^{-5} M, respectively). Their relative proportion is close to 1. Confirming previous data[2–7], the highly sensitive form was not detected in preparations from hearts maintained at a physiological calcium level. This heterogeneous population of functional $(Na^+ + K^+)$ATPases suggests that, in rat heart, the positive inotropic effect of low doses of ouabain *in vivo*[5–9] would act through the inhibition[9] of the enzyme activity highly sensitive to ouabain as detected *in vitro*.

Hearts isolated from male Wistar rats (240–300 g) were depleted of calcium or maintained at a normal calcium concentration (2 mM). In the former case, the heart was completely relaxed by perfusion either with an EDTA-containing relaxing buffer[10] (pCa 8) or with a Ca^{2+}-free Krebs–Ringer bicarbonate buffer (pCa 4.7)[11]. To maintain a physiological Ca^{2+} concentration, the perfusion was either omitted or performed with a Ca^{2+}-containing buffer. Sarcolemmal vesicles were isolated by differential centrifugations. The $(Na^+ + K^+)$ATPase was measured before and after unmasking the total activity with Na–SDS[12] or by reiterative freezings and thawings. As shown in Table 1, the preparation used in the present work is highly enriched in sarcolemmal vesicles with specific activities of $(Na^+ + K^+)$ATPase varying between 40 and 100 μmol of phosphate liberated per mg of protein per h. The sensitivity of $(Na^+ + K^+)$ATPase to ouabain was checked with 10^{-9} to 2×10^{-3} M ouabain (Boehringer) (prepared daily).

Native sarcolemmal vesicles isolated from relaxed hearts exhibited a two-step dose–response curve to ouabain (Fig. 1, curve *a*): 55±7% of the $(Na^+ + K^+)$ATPase activity was inhibited between 10^{-9} and 3×10^{-7} M ouabain, the half-maximal inhibition (IC_{50}) occurring with $1.2 \pm 0.2 \times 10^{-8}$ M ouabain. A plateau associated with 45±7% of the total activity was found between 3×10^{-7} and 10^{-5} M, 50% inhibition of this residual activity was obtained with $6 \pm 2 \times 10^{-5}$ M ouabain. This latter value is consistent with previous data on the insensitivity of rat heart $(Na^+ + K^+)$ATPase to ouabain[1–9,13] but involves only 45% of the enzyme activity.

A similar biphasic curve having the same proportion of the $(Na + K^+)$ATPase forms was obtained with sarcolemma vesicles subfractionated by centrifugation on sucrose[14] (Fig. 1, curve *a*). (Specific activity of $(Na^+ + K^+)$ATPase: 205±18 μmol phosphate h^{-1} mg^{-1}.)

Sarcolemmal vesicles from hearts maintained at a physiological Ca^{2+} level yielded a homogeneous population of $(Na^+ + K^+)$ATPase exhibiting a low sensitivity to ouabain (IC_{50} $4 \pm 2 \times 10^{-5}$ M) (Fig. 1, curve *b*) confirming all previous results[1–9,13]. Nevertheless, the yields in microsomal proteins and 5′-nucleotidase activity (a sarcolemma-bound marker[15]) were lower than those from hearts perfused with Ca^{2+}-free solutions, whereas the specific activity of the $(Na^+ + K^+)$ATPase remained constant (Table 1). These results are consistent with the observations of Zak *et al.*[16] who showed that the initial relaxed state of myofibrils facilitated the isolation of subcellular fractions in higher yield and with less destruction. However, as the specific activity of the $(Na^+ + K^+)$ATPase remained constant, one might suppose a conversion of the high affinity form into the low affinity one exhibiting the same molecular turnover. In considering both the specific activity and the protein yield, the total $(Na^+ + K^+)$ATPase activity in Ca^{2+}-containing hearts represented half that found in completely relaxed hearts. In the former case, the enzyme form highly sensitive to ouabain is lost or exists as a catalytically inactive conformation as suggested by Adams *et al.*[6].

Three more possibilities had to be excluded before concluding that it is the initial low Ca^{2+} level before preparation that reveals a $(Na^+ + K^+)$ATPase activity highly sensitive to ouabain.

First, a variable fraction of the vesicles used in this study may have an inside-out polarity; the curves may reflect differences in accessibility of ouabain to its internalized binding site or

Table 1 Distributions of proteins and enzyme activities in sarcolemmal vesicles

	Ca^{2+}-free perfused heart			Heart maintained at 2 mM Ca^{2+}		
	Protein yield	$(Na^+ + K^+)$ATPase	5′ Nucleotidase	Protein yield	$(Na^+ + K^+)$ATPase	5′ Nucleotidase
Sarcolemmal vesicles						
Native	1.2±0.1%	60±11 (36%)	5.4±1 (33%)	0.6±0.1%	40±10 (12%)	1±0.1 (3%)
Opened	/	100±9 (60%)	16±2 (96%)	/	95±15 (30%)	1.6±0.1 (5%)

Animals were killed by cervical dislocation, the heart was removed and either perfused with Ca^{2+}-free medium or maintained at a normal Ca^{2+} level (2 mM). Ca^{2+}-free hearts were perfused via coronary arteries for 1 min at room temperature with 5 ml of the ice-cold relaxing buffers[10,11]. Hearts at normal Ca^{2+} levels were either perfused in the same conditions with an ice-cold solution containing 2 mM $CaCl_2$, 25 mM KCl and 39 mM sodium tetraborate/HCl *p*H 6.8, or kept in ice for 5 min. The minced ventricles were homogenized at 4 °C in 30 ml of 20 mM sodium pyrophosphate (PP_i), 0.1 mM phenylmethanesulphonylfluoride, 10 mM Tris-HCl *p*H 7.8, with a Polytron PT 20 (2×0.5 s, setting 5). After stirring for 20 min at 4 °C, the suspension was made up to 120 mM KCl, 30 mM NaCl and 250 mM sucrose by adding concentrated solutions. The 500*g* 5-min supernatant was diluted to 120 ml of this isotonic and isoosmotic solution, adjusted to *p*H 6.8 with 40 mM imidazole–HCl, and centrifuged at 7,500*g* for 15 min and then at 31,000*g* for 30 min. The final pellet was washed in 50 mM PP_i, 135 mM NaCl, 5 mM $MgCl_2$ and 5 mM Tris-HCl *p*H 7.4 to eliminate myofibrils. The sarcolemmal vesicles were resuspended in 100 mM NaCl, 250 mM sucrose and 40 mM imidazole–HCl *p*H 6.8 and analysed for enzyme content. The $(Na^+ + K^+)$ATPase activity was assayed at 37 °C in a medium containing 10 μg sarcolemma protein[27], 4 mM $MgCl_2$, 100 mM NaCl, 10 mM KCl, 40 mM imidazole–HCl *p*H 7.4. The reaction was initiated with the addition of prewarmed ATP (final concentration 4 mM) and carried out over six time points to assess linearity. Aliquots were taken every 30 s and the released orthophosphate was measured[28]. The $(Na^+ + K^+)$ATPase activity was taken as that inhibited by 2×10^{-3} M ouabain. The activities of the $(Na^+ + K^+)$ATPase reported here represented 30–50% of total ATPase activities. 5′-Nucleotidase activity[15] was assayed for 1 h at 37 °C in a medium containing 20–100 μg protein ml^{-1}, 100 mM KCl, 10 mM $MgCl_2$, 5 mM AMP and 50 mM imidazole–HCl *p*H 7.4. This activity was taken as that insensitive to 10 mM potassium sodium tartrate[29] but that inhibited by 1 mM adenosine 5′-diphosphonate. Activities are expressed as μmol orthophosphate per mg of protein per h. Opened vesicles were obtained by pretreatment with Na–SDS[12] or by reiterative freezings and thawings: samples of freshly prepared sarcolemma vesicles (~0.1 mg) were frozen at −40 °C for 24 h, thawed, analysed for their enzyme content and frozen again. This procedure was performed twice. Values in parentheses give recovery of enzyme activity as % of total patent activity of homogenate. Whatever the pretreatment of the heart, the specific activities of $(Na^+ + K^+)$ATPase and 5′-nucleotidase in the ventricle homogenates were 2.1±0.5 and 0.2±0.06 μmol phosphate per h per mg, respectively. Note that in the intermediate fractions obtained along with the purification procedures, no significant $(Na^+ + K^+)$ATPase and 5′-nucleotidase activities could be measured.

variations in the proportion of inverted vesicles in the sarcolemma preparations. This possibility was excluded as a pretreatment of the vesicles, whatever their source, with Na–SDS or by reiterative freezings had no effect on the dose–response curves.

Second, the sensitivities of the $(Na^+ + K^+)$ATPase activity to ouabain seemed not to be due to drastic differences in the rates of drug binding to both native and opened vesicles. In the absence of ATP, either a 10-min or a 30-min equilibration period at 37 °C with varying doses of the drug led to the same inhibitions of the enzyme activity. In the presence of ATP, which favours ouabain binding[17], the amounts of phosphate liberated over the assays were linear with respect to time, suggesting that the reversible enzyme–ouabain equilibrium[2,3] was reached in less than 30 s (the shortest time used) even in the presence of 10 mM KCl.

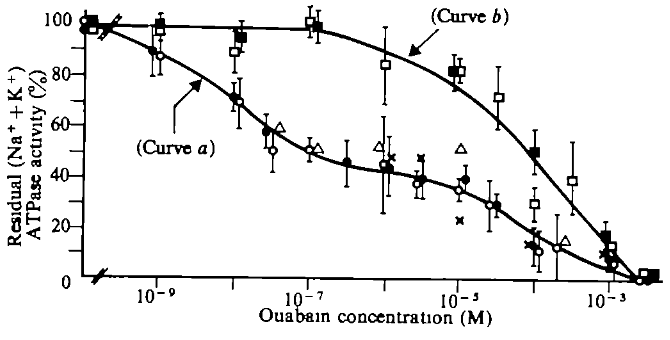

Fig. 1 Dose–response curves of $(Na^+ + K^+)$ATPase activity versus ouabain concentration. Curve *a*, Ca^{2+}-free perfused heart: native (●) and opened (○) sarcolemmal vesicles from ventricle, opened vesicles subfractionated on sucrose[14] (×), and opened vesicles (△) from isolated myocytes[11,28]. Curve *b*, heart maintained at 2 mM Ca^{2+}: native (■) and opened (□) sarcolemmal vesicles. The sensitivity of $(Na^+ + K^+)$ATPase was checked as follows. After incubation of sarcolemmal vesicles for 30 min at 37 °C with various amounts of ouabain in the ATP-free assay medium, ATP was added to initiate the enzymatic reaction. The activity at a given ouabain concentration (x) is: $[(\text{activity})^x - (\text{activity})^{2\times10^{-3}\text{ M}}/(\text{activity})^0 - (\text{activity})^{2\times10^{-3}\text{ M}}]\times100$. Whatever the pretreatment to which the heart was subjected, neither of our data showed net stimulation of the enzyme at any concentration. Each point represents the mean of 4–18 observations with bars denoting s.e.m. computed from percentage inhibition in each experiment. Each experiment was performed at least four times with at least four different sarcolemmal preparations.

Third, the enzyme form highly sensitive to ouabain could be associated with cell membranes from more than one cell type[18]. The experiments with sarcolemma vesicles from isolated myocytes[19] from relaxed hearts[11] ruled out this alternative explanation. The two-step inhibition by ouabain was the same as in sarcolemma preparations from the whole ventricle (Fig. 1, curve *a*). In isolated myocytes, Adams *et al.*[6] reported a high affinity binding site for ^{3}H-ouabain with a K_D of 1.2×10^{-7} M. Our process of isolation may have changed the properties of the enzyme in relation to the effect of the glycoside, that is, an increased sensitivity. However, the disparity with our data may be due to calcium loading as a result of the long-lasting incubation of the myocytes at 37 °C with 0.5 mM Ca^{2+}. This would lead to a situation different from the physiological conditions (intact heart, 2 mM external calcium and 10^{-5}–10^{-7} M internal free Ca^{2+}) and the conditions used here (sarcolemma vesicles, 3×10^{-6} M free Ca^{2+}). Thus, the dose–response curve for the low ouabain concentration reported by Adams *et al.*[6] would represent the sum of both high and low sensitivity enzyme forms in an unknown proportion depending on the internal Ca^{2+} pools and/or the size of the rapidly exchanging calcium stores[20].

These parameters would control the expression of the high sensitivity enzyme form. Such an effect of Ca^{2+} on the effects of ouabain is consistent with the different relationships between ouabain and the systolic (10^{-5} M internal free Ca^{2+}) and diastolic (10^{-7} M internal free Ca^{2+}) tensions in rat heart[7]. Further, the experiments of Carrier *et al.*[21] show that in guinea pig heart preparations, the relative inotropic action of ouabain is much larger when the Ca^{2+} store is small than when it is large. A direct effect of millimolar external Ca^{2+} levels on the expression of the two enzyme forms cannot be excluded but is very unlikely since our observations are consistent with the biphasic inotropic action of ouabain (10^{-8}–10^{-6} M and 10^{-4} M) in isolated rat hearts perfused with 1.4 mM Ca^{2+} (ref. 22).

There is some evidence that two components could be involved in the inotropic effect of ouabain *in vivo*[5–8,22] as if the high- and low-affinity ouabain-binding sites detected in rat heart sarcolemmal vesicles were two $(Na^+ + K^+)$ATPase isozymes[6] being inhibited by the drug in a concentration range between 3×10^{-8} M and 10^{-3} M[7]. Alternatively, a single class of enzyme molecules may express one or two sensitivities to ouabain depending both on the Ca^{2+} concentrations[23,24] and on the interactions with other membrane proteins[24,25].

The present results provide further insight into the therapeutic mechanisms of ouabain[26]: the high sensitivity enzyme form might represent the physiological site responsible for the positive inotropic effect of low concentrations of ouabain *in vivo*.

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Minute virus of mice inhibits cell transformation by simian virus 40

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Certain paroviruses have been reported to inhibit spontaneous[1] or virus-induced[2] tumorigenesis in rodents; the mechanisms of this antineoplastic activity is unknown. We have investigated the ability of the minute virus of mice (MVM), a non-defective parvovirus[3], to interfere with the *in vitro* transformation of mouse cells by simian virus 40 (SV40). We used variants of BALB/c 3T3 cells, denoted PVR, selected for their resistance to the lytic effect of MVM. We report here that inoculation of SV40-infected PVR cells with MVM reduced dramatically the yield of transformed clones. Moreover, stable SV40 transformants of PVR cells lost up to 85% of their ability to grow in soft agar after MVM infection. Our results indicate that the antineoplastic effect of parvoviruses can be simulated in cell cultures and may involve a direct and selective toxic effect of these viruses on malignant cells or their precursors.

In mature animals, parvoviruses interfere with the development of tumours but are, in general, innocuous for normal cells regardless of their mitotic activity[3]. It is unclear whether this selective inhibition of oncogenesis depends on complex events occurring in whole animals such as immunological, hormonal or interferon induction responses. We therefore investigated whether the antineoplastic activity of parvoviruses could be reproduced and analysed *in vitro* in the absence of these physiological processes. An inhibition of the oncogenesis of adenovirus[4]- or herpes simplex virus[5]-transformed cells was achieved by exposing *in vitro* cultures of these cells to adeno-associated virus (AAV), a defective parvovirus, before inoculation into animals. Adenovirus and herpes simplex virus are the only two viruses that can serve as helper for the replication of AAV. Thus, it is questionable whether the antineoplastic activity of AAV results from its relationship to the helper virus or from a direct interaction between the parvovirus and the transformed cell *per se*. We tested the latter possibility by investigating whether the non-defective parvovirus MVM interfered with *in vitro* cell transformation by SV40, a virus without discernible helper activity for parvoviruses.

The BALB/c 3T3 mouse cell line[6] was used as it is highly susceptible to SV40 transformation. These cells are normally sensitive to MVM lytic infection, as are other established cultures of mouse fibroblasts. To mimic the *in vivo* situation we isolated from BALB/c 3T3 cells a series of variant clones resistant to MVM infection. These clones were denoted PVR (parvovirus-resistant). Typical properties of PVR cells are illustrated in Table 1. The PVR cells resisted viral attack as shown by both high cell survival and low production of infectious progeny virus. In contrast, PVR cells did not differ significantly from the permissive parent cells with respect to MVM uptake (data not shown), suggesting that their resistance occurred at an intracellular step of MVM replication.

Table 2 (columns *a* and *b*) shows that the PVR strains tested were susceptible to transformation by the oncogenic virus SV40 as measured by the ability of SV40-infected cells to form colonies in soft agar. The transformation frequency of PVR cells inoculated with SV40 (100 plaque-forming units (PFU) per cell) ranged from 1 to 5% and was equivalent to that of parental BALB/c 3T3 cells. The selection of PVR cells therefore had no detectable effect on their susceptibility to *in vitro*

Table 1 Susceptibility of BALB/c 3T3 and PVR-1 cells to MVM infection

Multiplicity of infection (PFU per cell)	Surviving fraction relative to uninfected cells		Fraction of inoculated cells producing infectious centres	
	BALB/c 3T3	PVR-1	BALB/c 3T3	PVR-1
1	$<10^{-3}$	0.99	0.41	0.008
10	$<10^{-3}$	0.90	–	–
100	$<10^{-3}$	0.78	0.66	0.05

The PVR-1 strain was cloned from a population of BALB/c 3T3 cells[4] surviving two successive infections with 100 PFU of MVM per cell. Cells from the parental BALB/c 3T3 line and from the PVR-1 strain were inoculated with increasing amounts of MVM. After 1 h, the inoculum was removed and extracellular virus was inactivated by incubation for 3 h in medium containing rabbit anti-MVM serum[7] (given by P. Tattersall). Cultures were then collected and processed for cell survival and infectious centre assays. Cell survival was measured by colony formation on plates and is expressed relative to uninfected cells. The plating efficiencies of uninfected BALB/c 3T3 and PVR-1 cells were 0.35 and 0.27, respectively. The fraction of productively infected cells was measured by plaque formation after seeding of MVM-inoculated cells on to appropriate indicator cells[7]. The resistance of PVR cells to MVM attack was maintained during continuous subculturing for at least 1 yr.

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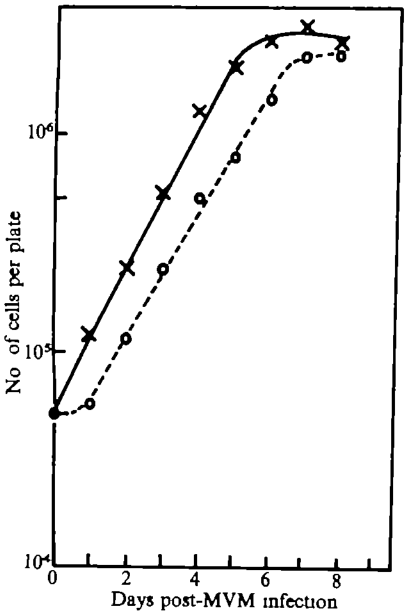

Fig. 1 Effect of MVM on the growth of monolayer cultures of SV40-transformed PVR-1 cells. SV40-transformed PVR-1 cells in 60-mm dishes were inoculated with MVM (100 PFU per cell; (O) or mock-infected(×). Cultures were further incubated in liquid medium containing rabbit anti-MVM serum[7] and collected at various intervals. Cells were counted with a haemocytometer.

transformation. Clearly, MVM reduced dramatically the yield of *in vitro* transformation of PVR cells previously infected by SV40 (Table 2, columns *c* and *d*). In order to identify which step of the transformation process is the target for MVM, we isolated stable SV40 transformants of PVR-1 cells and tested their sensitivity to MVM. Table 3 shows that MVM infection decreased markedly the viability of SV40-transformed PVR-1 cells as measured by their cloning efficiency on plastic plates. As SV40 transformation did not increase the plating efficiency of PVR-1 cells (see Tables 1 and 3), this effect can be accounted for, not by the reversion of the transformed phenotype, but rather by a cytotoxic action of MVM on transformed cells. Although 75% of the transformed PVR-1 cells were killed after inoculation with MVM at a multiplicity of 100 PFU per cell, only 16% of these cells supported a productive MVM infection (Table 3). However, the yield of infectious centres was 3–6 times greater for SV40-transformed (Table 3) than for normal (Table 1) cells. Thus, SV40 transformation was apparently associated with a greater sensitivity to the cytotoxic effect of MVM although only a very small fraction of transformed cells was fully permissive to this virus. The abortion of the productive infection in a fraction of the sensitive cells is a common, yet quantitatively variable, feature of parvovirus replication, as illustrated in Table 1 for BALB/c 3T3 cells. Table 3 also indicates that the cloning efficiency of stable SV40 transformants of PVR-1 cells in soft agar was reduced by 85% after infection with MVM at a multiplicity of 100 PFU per cell. As SV40-inoculated PVR-1 cells were prevented from forming clones in soft agar to a similar extent by MVM co-infection (Table 2), the data suggest that the presence of MVM during the establishment of the transformed phenotype is not essential for the inhibition of *in vitro* cell transformation.

Table 2 Effect of MVM on *in vitro* transformation of PVR cells by SV40

	Cloning efficiency in soft agar(%)			*d*, MVM inhibition of SV40 transformation
Cells	*a*, No virus	*b*, +SV40	*c*, +SV40 +MVM	(%)
PVR-1	$<5\times10^{-5}$	3.6	0.38	89.5
PVR-2	$<5\times10^{-5}$	2.4	0.37	84.2
PVR-3	$<5\times10^{-5}$	1.7	0.05	97.1

PVR-1, -2 and -3 strains were cloned from a population of BALB/c 3T3 cells surviving MVM infection and achieved similar levels of resistance to MVM (see Table 1). PVR strains were tested for their anchorage-independent growth ability, as measured by the formation of foci in soft agar[9]. *a*, Uninfected cells; *b*, cells inoculated with SV40 (100 PFU per cell) and propagated for six generations before suspension in agar-containing medium; *c*, same as *b*, except that cells were superinfected with MVM (100 PFU per cell) 24 h after SV40 inoculation. *a* and *b* series underwent mock infections. Intervals between SV40 and MVM infections ranging from 0 to 24 h were tested and gave essentially the same results (data not shown). Foci were counted 4 weeks after suspension in soft agar and were of similar size in all series. Incubation of MVM-treated cells for longer periods did not increase cloning efficiency.

The effect of MVM on the growth of monolayer cultures of SV40-transformed PVR-1 cells was studied in conditions that prevented secondary MVM infections. Figure 1 shows that MVM caused a delay in the proliferation of transformed PVR-1 cells. This lag was not detectable for non-transformed cells (data not shown) and probably reflects the killing of a fraction of the cells as a result of MVM infection (Table 3). SV40-transformed PVR-1 cells that survived MVM inoculation were not intrinsically resistant to MVM as they could be superinfected and showed the same growth delay as the parental culture (data not shown). Cell survivors contributing to the proliferation of the culture after the 1-day lag period did not differ markedly from uninfected cells with respect to their saturation density and growth rate (Fig. 1). It can be calculated from Fig. 1 that the generation time of MVM-infected cells was ~24 h, less than 1.5 h longer than for uninfected cells. Thus, the major restriction imposed by MVM on SV40-transformed cells seems to occur at the level of cell viability rather than growth properties. Recently, opposite conclusions were reached for the action of the defective parvovirus AAV on adenovirus-transformed cells. This discrepancy could be due to the fact that adenovirus is a helper for AAV replication. This interrelationship imposes specific restrictions on the expression of both viruses after AAV infection of adenovirus-transformed cells[4] and might account for the selective interference of AAV with the oncogenicity of its helper viruses[4,5]. Thus, the mechanisms underlying the inhibition of *in vitro* transformation might be at least partly different for defective AAV[4,8] and for autonomous MVM parvoviruses.

The results presented here indicate that one pathway of parvoviral surveillance against cancer might involve a direct and selective inactivation of transformed cells by these viruses. The privileged interaction of parvoviruses with transformed cells strongly suggests that transformation sensitizes cells that are normally resistant to virus infection, although a preferential transformation of pre-existing parvovirus-sensitive cells cannot be ruled out. It has been shown recently that the cellular permissivity to lytic parvovirus replication is controlled by developmentally regulated host factors[7]. The relevance of our

Table 3 Effects of MVM on SV40-transformed PVR-1 cells

			Colony formation in soft agar	
Multiplicity of infection (PFU per cell)	Cell surviving fraction	Fraction of productively infected cells	Cloning efficiency (%)	% Inhibition
0	1	0	7.8	0
0.1	—	—	5	36
1	0.52	0.05	4.1	48
10	0.32	—	2.1	74
100	0.25	0.16	1.2	85

A strain of transformed cells was derived from a clone of SV40-infected PVR-1 cells growing in soft agar (Table 2, column *b*). These cells expressed SV40 T antigen as measured by immunostaining (data not shown). SV40-transformed PVR-1 cells were inoculated with increasing amounts of MVM. Infected cells were then processed for cell survival and infectious centre assays (see Table 1 legend) or incubated for 5 h and suspended in soft agar for transformation assay (see Table 2). Viability and virus production were measured in the same experiment as for normal cells (Table 1). The plating efficiency of SV40-transformed PVR-1 cells was 0.12. The time of appearance and the size of foci in soft agar were not affected by MVM infection experimental system to the situation *in vivo* rests on the assumption that the expression of such a factor(s) is limiting in PVR cells as it is unknown why these selected cells fail to replicate MVM. Yet our data raise the possibility that changes in cellular gene expression triggered by neoplastic transformation might lift barriers to parvovirus replication, thereby making malignant cells a preferential target for the cytotoxic action of these viruses. Use of our *in vitro* system for the biochemical analysis of the MVM life cycle should elucidate these barriers. Such a system provides a means of studying the molecular mechanism of the parvoviral antineoplastic activity.

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Oncogenes in solid human tumours

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The identification and isolation of oncogenes capable of inducing malignant transformation on transfection into rodent cells from human tumour cells has opened the possibility of deciphering the processes involved in human carcinogenesis. With the exception of three human lymphomas[1], the human transforming genes so far identified have been detected in established tumour cell lines[1–8], raising the possibility that they might have been activated during *in vitro* manipulation of the cells. However, we report here that unmanipulated human solid tumours, including carcinomas of the colon (two), lung and pancreas and an embryonal rhabdomyosarcoma, also contain dominant transforming genes. The carcinomas of the lung and pancreas and the rhabdomyosarcoma possessed a common oncogene which, like that isolated from human LX-1 lung carcinoma cells[9], shares sequences with the *onc* gene of the Kirsten strain of murine sarcoma virus (MSV). We also show that several other human tumour cell lines, including those established from carcinomas of the colon (A2233), lung (A427 and A2182), gall bladder (A1604) and urinary bladder (A1698), possess the same oncogene. Thus a variety of human tumours, regardless of their clinical manifestations, contain a common transforming gene.

We have isolated high-molecular weight DNA from a variety of surgically removed solid human tumours, some of which have been stored at −70 °C for up to 5 yr. These human DNAs were used to transfect NIH 3T3 mouse cells by the calcium phosphate precipitation technique[10,11]. Foci of morphologically transformed NIH 3T3 cells were picked by the cloning cylinder technique and cloned as single cells in semi-solid media. The clonal cells were grown to mass culture and their DNAs analysed for the presence of human repetitive sequences[2] (Table 1). About 15% of the human solid tumours tested (5 out of 28) which included carcinomas of the colon (two), lung and pancreas and an embryonal rhabdomyosarcoma from a 1-yr-old patient, were found to contain dominant oncogenes capable of transforming NIH 3T3 cells. Transformation efficiencies (0.05–0.1 focus per μg of donor DNA per 2×10^5 NIH 3T3 cells) were about fivefold lower than those observed with T24 bladder carcinoma DNA, but comparable with those obtained with DNAs isolated from other human tumour cell lines[8]. Interestingly, DNA isolated from normal tissue of the patient, whose pancreatic carcinoma exhibited an oncogene, failed to induce transformation of NIH 3T3 cells in the same experimental conditions.

After three cycles of transfection, human DNA sequences encompassing the corresponding oncogenes present in each of the above solid human tumours were identified by Southern blot analysis using a probe specific for human repetitive sequences. Representative third-cycle transformants derived from a colon carcinoma of a 66-yr-old female, exhibited two common *Eco*RI-cleaved DNA fragments of about 10 and 7 kilobase pairs (kbp) (Fig. 1*A*). NIH 3T3 transformants derived from a second colon carcinoma obtained from the ascending portion of the colon of a 57-yr-old male patient also possessed two common *Eco*RI-cleaved human DNA fragments, although of different sizes (25 and 4 kbp; Fig. 1*B*). Southern blot analysis of these third-cycle transformants with other restriction endonucleases such as *Hin*dIII and *Bam*HI revealed different patterns of human DNA fragments (data not shown), suggesting that these two colon carcinomas contain different transforming genes.

When similar studies were performed with representative third-cycle transformants derived from the lung and pancreas carcinomas and from the rhabdomyosarcoma, a different picture emerged. All the transformants tested exhibited a common pattern of *Eco*RI-cleaved human DNA fragments of about 6.8, 6.7, 4.4, 4.0, 3.0 and 2.4 kbp (Fig. 1*C*). In analogous experiments, these transformants were found to possess three common *Hin*dIII-cleaved human DNA fragments of about 18.0, 12.2 and 3.4 kbp (data not shown). These results indicate that three different solid human tumours possess the same, or a highly related, oncogene.

We have also detected the presence of transforming genes in various human tumour cell lines including those established from carcinomas of the colon (A2233), lung (A427 and A2182), gall bladder (A1604) and urinary bladder (A1698 and T24) as well as from a fibrosarcoma (HT-1080). When the human DNA fragments carrying the corresponding oncogenes were characterized by submitting the DNAs of representative third-cycle NIH 3T3 transformants to Southern blot analysis, we observed that colon carcinoma A2233, lung carcinomas A427 and A2181, gall bladder carcinoma A1604 and urinary bladder carcinoma A1698 cells also contained the same pattern of human DNA fragments found in the oncogene present in the three solid human tumours described above (data not shown). Thus of the 12 human tumours (5 solid tumours and 7 established tumour cell lines) in which we have identified dominant transforming genes, 8 (67%) possess the same, or a highly related, oncogene.

Recently, Perucho *et al.* have reported that three human tumour cell lines established from lung and colon carcinomas contained the same transforming gene[6]. Moreover, this gene was indistinguishable from that previously identified by Murray *et al.* in SW480 colon carcinoma cells[5]. Close examination of the pattern of *Eco*RI-cleaved fragments of this lung/colon carcinoma oncogene and that depicted in Fig. 1*C* for the oncogene identified here in eight different tumours strongly suggests that they are the same oncogene.

It has been recently demonstrated that a human transforming gene isolated from T24 and EJ bladder carcinoma cells[8,12,13] is an activated form of the normal human homologue of the *onc* genes of the BALB and Harvey strains of murine sarcoma viruses (MSV)[9,14,15]. Furthermore, Der *et al.* have shown that the oncogene present in LX-1 human lung carcinoma cells contains a 3.0-kbp *Eco*RI fragment highly related to *kis*, the *onc* gene of the Kirsten strain of MSV[9]. Thus, we decided to

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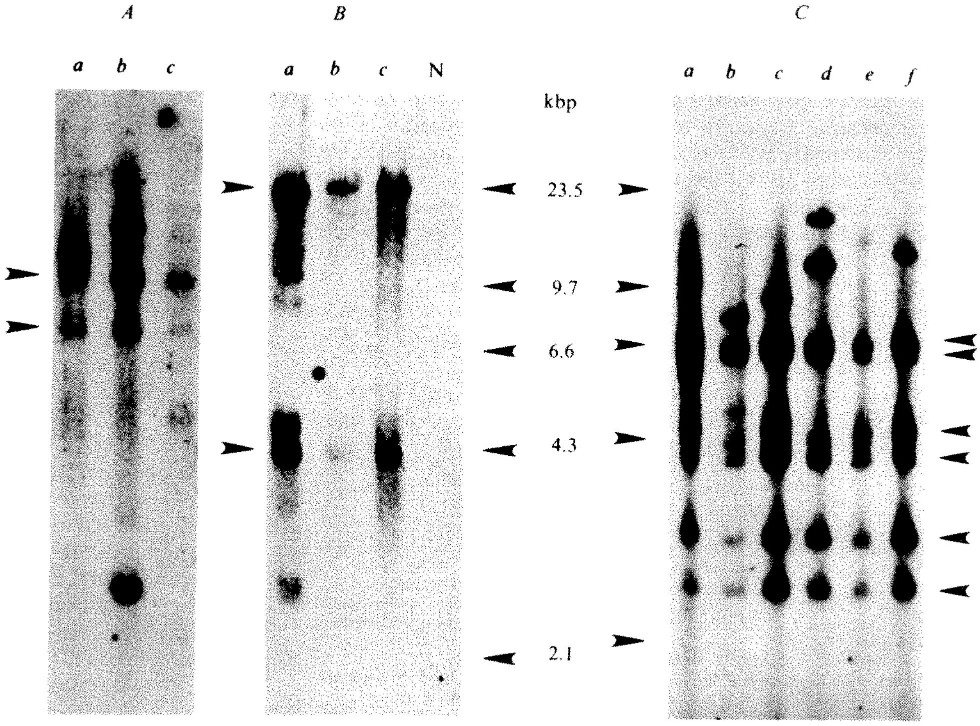

Fig. 1 Southern blot analysis of human repetitive sequences in third-cycle NIH 3T3 transformants derived from solid human tumours. 20 μg of high-molecular weight DNA isolated from: *A*, *a–c*, representative third-cycle transformants obtained by transfection with DNA isolated from colon carcinoma 1665; *B*, *a–c*, representative third-cycle transformants obtained by transfection with DNA isolated from colon carcinoma 2033; N, control NIH 3T3 mouse cells; *C*, representative third-cycle transformants obtained by transfection with DNA isolated from rhabdomyosarcoma 1085 (*a*, *b*), lung carcinoma 1615 (*c*, *d*) and pancreatic carcinoma 1189 (*e*, *f*) were digested with 20 U of *Eco*RI for 1 h at 37 °C, electrophoresed (18 h at 30 V) in horizontal agarose (0.7% w/v) gels and blotted to nitrocellulose filters as described by Southern[24]. Filters were hybridized in stringent conditions (50% formamide, 5 × SSC and 42 °C) for 44 h to 4×10^7 c.p.m. of nick-translated high-molecular weight human DNA. Hybridized blots were exposed to Kodak XR-5 film at −70 °C in the presence of intensifier screens for 2 days. Co-electrophoresed DNA fragments of *Hin*dIII-digested λ c1857 DNA served as size standards (labelled in kbp). Migration of *Eco*RI-cleaved human DNA fragments shared by third-cycle transformants derived from either *A*, colon carcinoma 1665; *B*, colon carcinoma 2033; or *C*, rhabdomyosarcoma 1085, lung carcinoma 1615 and pancreatic carcinoma 1189 are indicated by arrows.

investigate whether the oncogene present in the rhabdomyosarcoma, lung and pancreatic carcinomas, and in A2233 colon, A427 and A2182 lung, A1064 gall bladder and A1698 urinary bladder carcinoma cell lines also contained sequences related to retroviral transforming genes. DNAs isolated from representative third-cycle transformants derived from each of the above tumours were digested with *Eco*RI and submitted to Southern blot analysis for the presence of retroviral *onc* gene sequences. Radioactive probes included those specific for v-*abl* (Abelson murine leukaemia virus), v-*bas* (BALB-MSV), v-*fes*ST (Snyder–Theilen strain of feline sarcoma virus), v-*mos* (Moloney-MSV), v-*myb* (avian myeloblastosis virus), v-*myc* (avian MC29 virus), v-*kis* (Kirsten-MSV), v-*sis* (simian sarcoma virus) and v-*src* (Rous avian sarcoma virus)[15].

The *kis* probe specifically detected the presence of two *Eco*RI-cleaved DNA fragments of 6.8 and 3.0 kbp in the genomes of each of the NIH 3T3 transformants tested (Fig. 2). Neither of these DNA fragments was found in control NIH 3T3 cells, indicating that they were derived from c-*kis*, the human homologue of the *onc* gene of Kirsten-MSV[16]. None of the other retroviral probes used detected human DNA sequences present in these NIH 3T3 transformants, with the exception of v-*bas* which hybridized weakly to the 3.0-kbp *Eco*RI DNA fragment of human c-*kis*[16,17].

To determine whether the retrovirus-related sequences of this human oncogene were expressed, we analysed NIH 3T3 transformants for the presence of proteins antigenically related to p21, the gene product of Kirsten-MSV[18]. Radioactively

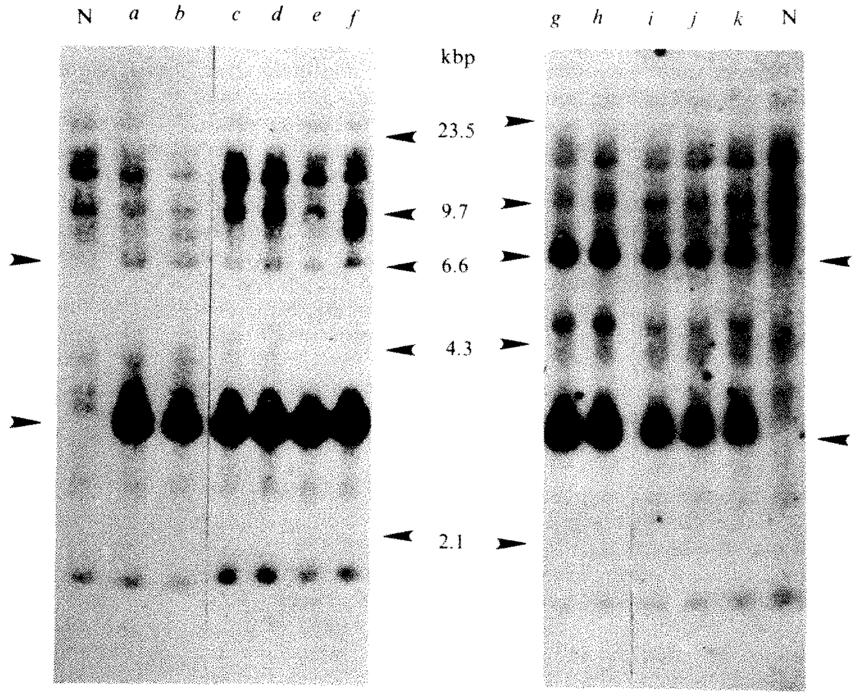

Fig. 2 Detection of sequences related to the *onc* gene of Kirsten-MSV in NIH 3T3 cells transformed by human tumour DNA. 20 μg of *Eco*RI-cleaved DNAs isolated from third-cycle transformants derived from rhabdomyosarcoma 1085 (*a*, *b*), lung carcinoma 1615 (*c*, *d*), pancreatic carcinoma 1189 (*e*, *f*), A2233 colon carcinoma cells (*g*), A427 lung carcinoma cells (*h*), A2182 lung carcinoma cells (*i*), A1604 gall bladder carcinoma cells (*j*) and A1698 bladder carcinoma cells (*k*) as well as from control NIH 3T3 cells (N), were blotted to nitrocellulose filters and hybridized to 4×10^7 c.p.m. of nick-translated Kirsten-MSV DNA clones 4E *Pvu*II (ref. 25) or pHiHi-3 (ref. 16) as described in Fig. 1 legend. Co-electrophoresed DNA size standards were those described in Fig. 1 legend. Migration of the 6.8- and 3.0-kbp *Eco*RI-cleaved DNA fragments of the human c-*kis* gene[16] is indicated by arrows.

labelled cell extracts of representative third-cycle transformants derived from each of the three human solid tumours and from several of the human tumour cell lines known to possess this gene (Table 2) were immunoprecipitated with antisera obtained from BALB-MSV-induced tumour-bearing rats. These antisera contain broadly reactive antibodies capable of recognizing not only the gene products of BALB-, Harvey- and Kirsten-MSVs, but also those coded for by the corresponding mouse and human homologue c-*onc* genes[15]. All the transformants tested exhibited elevated levels (about four- to eightfold higher than NIH 3T3 control cells as determined by densitometric analysis) of Kirsten-MSV p21-related proteins of molecular weights (MWs) 20,000–22,000 (Fig. 3). The different molecular weights exhibited by these proteins suggest the existence of some polymorphism in the human c-*kis* sequences. In one case (Fig. 3*G*), a third-cycle transformant derived from a pancreatic carcinoma exhibited an unusual Kirsten-MSV-related protein of MW 27,000. Because all other NIH 3T3 transformants derived from this tumour contained 21,000-MW proteins, it is possible that certain genetic alterations may occur during transfection. These results indicate that the observed Kirsten-MSV-related proteins represent the translational products of the transfected human oncogenes, thus providing further evidence that the c-*kis* sequences of this oncogene play a part in malignant transformation.

Previous studies have shown that a small percentage of human tumour cell lines contained oncogenes capable of inducing malignant transformation of NIH 3T3 mouse cells[1–8]. We have demonstrated here that similar results can be obtained using solid human tumours. About 15% of the tumour specimens whose DNAs were tested in transfection assays were shown to contain dominant transforming genes. The relatively high transformation efficiencies observed with these solid human tumour DNAs implies the presence of oncogenes in most of the cells present in these tumours. These results support the hypothesis that these oncogenes have played a part in the development of these neoplasias.

Molecular characterization of dominant transforming genes present in 12 different human tumours has revealed the frequent detection of an oncogene containing sequences related to the *onc* gene of Kirsten-MSV. In fact, this oncogene was detected in eight tumours, including a rhabdomyosarcoma and carcinomas of several organs such as colon, lung, gall bladder and urinary bladder. Other investigators have detected the same, or a closely related, oncogene in five additional lung and colon carcinoma cell lines[4,5,9]. These findings, together with the recent demonstration that the T24 and EJ bladder carcinoma oncogene is a transforming allele of the normal human c-*has*/*bas* gene[9,14,15], have posed the question of why most of the transforming genes present in human carcinomas are related to the c-*has*/*bas*/*kis* gene family. It could be argued that

Table 1 Summary of DNAs isolated from naturally occurring human tumours tested for transformation of NIH 3T3 mouse cells in transfection assays

Type of tumour	Total tested	Positive in transfection assays
Carcinomas		
Bladder	2	0
Breast	2	0
Colon	2	2
Kidney	3	0
Lung	5	1
Ovary	4	0
Pancreas	2	1
Sarcomas		
Fibrosarcoma	4	0
Osteosarcoma	2	0
Rhabdomyosarcoma	2	1

Human tumours were thawed and the necrotic tissues removed with a scalpel. Tumorous tissue was finely minced with the aid of razor-sharp scissors, disrupted in a loose-fitted Dounce, washed three times with phosphate-buffered saline and incubated for 16 h at 37 °C with 200 μg ml^{-1} of self-digested pronase in the presence of 0.2% SDS. High-molecular weight DNA was isolated by successive extractions with 1 vol of phenol, 1 vol of phenol/chloroform/isoamylalcohol (25 : 24 : 1) and chloroform/isoamylalcohol (24 : 1) followed by precipitation with 2 vols of ethanol. These DNAs (30 μg per plate, 16 plates in 4 different transfection experiments) were used to transfect NIH 3T3 cells by the calcium phosphate precipitation technique[10], as described previously[8,11].

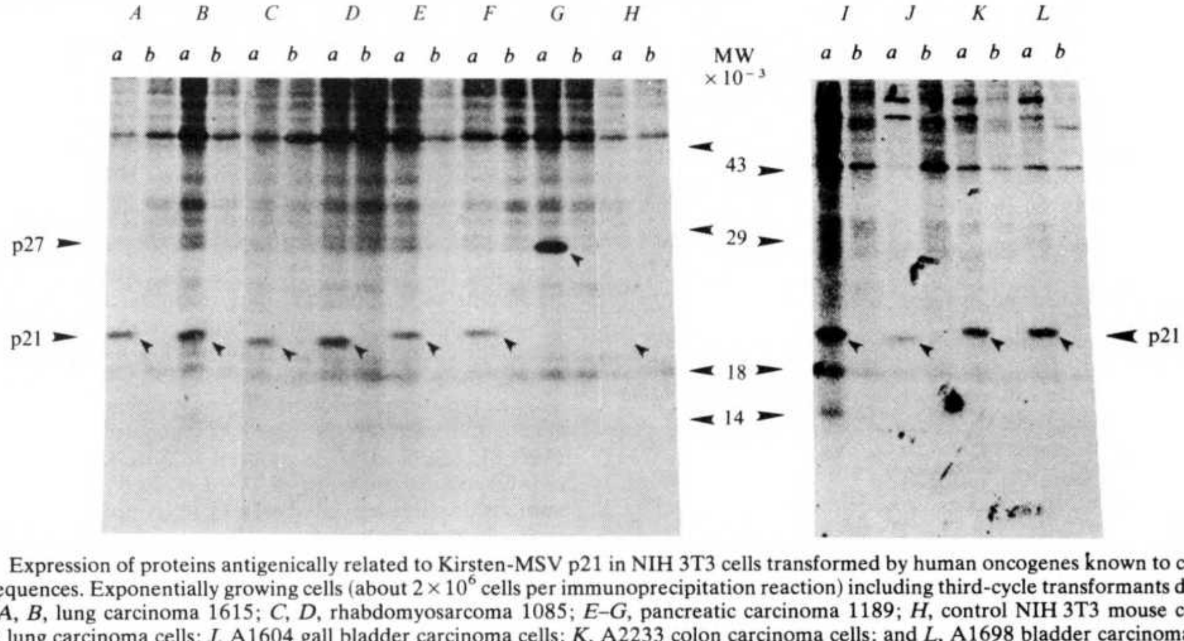

Fig. 3 Expression of proteins antigenically related to Kirsten-MSV p21 in NIH 3T3 cells transformed by human oncogenes known to contain c-*kis* sequences. Exponentially growing cells (about 2×10^6 cells per immunoprecipitation reaction) including third-cycle transformants derived from: *A*, *B*, lung carcinoma 1615; *C*, *D*, rhabdomyosarcoma 1085; *E–G*, pancreatic carcinoma 1189; *H*, control NIH 3T3 mouse cells; *I*, A2182 lung carcinoma cells; *J*, A1604 gall bladder carcinoma cells; *K*, A2233 colon carcinoma cells; and *L*, A1698 bladder carcinoma cells, were metabolically labelled with ^{35}S-methionine as previously described[26]. Post-microsomal fractions were incubated for 1 h at 4 °C with 0.01 ml of sera obtained from: *a*, BALB-MSV-induced tumour-bearing rats[15] or *b*, uninfected normal rats. Immunoprecipitates were collected with the aid of *Staphylococcus aureus* protein A bound to Sepharose 4B beads and disrupted in the presence of 1% SDS and 2% 2-mercaptoethanol by incubating them at 95 °C for 2 min. Denatured proteins were analysed by electrophoresis (18 h at 100 V) in SDS-polyacrylamide (14% w/v) gels. Electrophoresed gels were dried, fluorographed[27] and exposed to Kodak XR-5 films for 5 days at −70 °C. Co-electrophoresed molecular weight markers included ovalbumin (43,000), carbonic anhydrase (29,000), lactoglobulin (18,000) and lysozyme (14,000). Migration of proteins antigenically related to Kirsten-MSV p21 is indicated by arrows.

Table 2 Summary of human oncogenes detected in our laboratory

Type of tumour	Source of DNA	Ref	Oncogene	Relationship with retroviral *onc* genes
Carcinomas				
Bladder	T24 cells	21	*onc* A	*has, bas*
Bladder	A1698 cells		*onc* B	*kis*
Colon	Solid tumour 1665		*onc* C	—
Colon	Solid tumour 2033		*onc* D	—
Colon	A2233 cells		*onc* B	*kis*
Gall bladder	A1604 cells		*onc* B	*kis*
Lung	A2182 cells	8	*onc* B	*kis*
Lung	A427 cells	22	*onc* B	*kis*
Lung	Solid tumour 1615		*onc* B	*kis*
Pancreas	Solid tumour 1189		*onc* B	*kis*
Sarcomas				
Fibrosarcoma	HT-1080 cells	23	*onc* E	—
Rhabdomyosarcoma	Solid tumour 1085		*onc* B	*kis*

Human tumour cell lines A1604, A1698, A2182 and A2233 were established by S.A.A. Their human origin and independent identity have been determined by karyologic and isoenzyme (ADA, G-6-PD, LDH, PGD, PGM_1 and PGM_3) analysis. *onc* A, *onc* B and so on is an arbitrary designation used solely to indicate genetically distinct oncogenes. Retroviral *onc* genes tested· *abl, bas, fes, mos, myb, myc, kis, sis* and *src.*

transfection protocols favour the identification of a particular type of human oncogene that preferentially transforms NIH 3T3 mouse cells. However, Lane *et al.*, using the same assay cells, have shown that none of nine oncogenes present in lymphoid human tumours appeared to be a member of the *has/bas* gene family[9]. Moreover, these authors have identified different transforming genes in neoplasias representing different stages of lymphocyte differentiation[1]. Thus, it is possible that the c-*has/bas*-related oncogenes may be preferentially, although not specifically, activated in tumours of epithelial origin.

Shilo and Weinberg have recently shown that several 3-methylcholanthrene-transformed mouse cell lines may contain the same oncogene[19]. Interestingly, all oncogenes so far detected in carcinomas of the lung appeared to be the same (Table 2; refs 5, 6, 9). Although no definitive aetiology has been established for any form of human cancer, most lung carcinomas have been associated with smoking[20]. These observations are consistent with the hypothesis that neoplasms induced by certain carcinogens may be associated with the activation of a particular cellular oncogene.

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Regulatory mutants of the maize *Adh1* gene caused by DNA insertions

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Transposable elements in maize can induce changes in the timing of gene expression and the quantity of gene product[1,2]. The timing of expression of the *bronze* locus has been shown genetically to be affected by insertion of the transposable element *Ds* (dissociation)[3]. Molecular evidence has demonstrated that insertion of *Ds* at another locus, *shrunken-1*, disrupts the DNA sequence as expected of an insertion[4,5]. Here we report quantitative changes in gene expression resulting from the insertion of a 1.5-kilobase (kb) DNA element at an affected gene. The alcohol dehydrogenase-1 (*Adh1*) mutants described here, selected via allyl alcohol-resistant pollen grains[6], were recovered from a genetic background containing Robertson's mutator (*Mu*)[7]. We suggest that the *Mu* genetic background caused the DNA insertion at *Adh1*, and that the insertion assumed some regulatory function in the control of *Adh1* expression.

The *Adh1* alleles considered here, and their products, have been described elsewhere[8,9]. Their genealogies and characteristics are summarized in Fig. 1. *Adh1-S3034* (*S3034*) was induced in an *Adh1-S* (*S*) progenitor allele as a consequence of crossing an *S* line to a line carrying *Mu* and an electrophoretically distinct *Adh1* allele. The variant *Adh1* in the *Mu* line allowed unambiguous identification of *S* alleles among progeny, and recovery of marker genes from both parental lines ensured that *S3034* was not the product of pollen contamination.

S3034 is expressed in all organs normally exhibiting the *S* gene product, but at a level 40% that of the progenitor. It is 100-fold less stable than ethylmethane sulphonate (EMS)-induced mutants as judged from pollen samples stained for alcohol dehydrogenase (ADH) activity. Genetically unstable derivative alleles having even less ADH1 were selected from *S3034* lines as shown in Fig. 1: *S3034b* expresses 13% of the ADH1 activity seen in *S*, and *S3034a* expresses none.

ADH1 polypeptides are synthesized *de novo* by roots in response to anaerobic stress[10,11]. Gerlach *et al.*[12] have demonstrated, and we have confirmed in our lines (data not shown), that increased levels of ADH1 produced in anaerobic conditions are accompanied by increased levels of ADH1 RNA. Steady-state RNA levels are reached within 6 h.

We compared polyadenylated ADH RNA levels in roots from homozygotes of *S, S3034, S3034a* and *S3034b* after subjecting seedlings to 12 h of anaerobic stress. Figure 2 shows the results of a Northern blot in which equal amounts of polyadenylated RNA from the four homozygotes were hybridized to the cDNA probe pZmL84 (see Fig. 4 legend for descriptions of the probes). Results from RNA dot-blots of the same samples are shown below the corresponding gel tracks. 'Dots' were cut from filters for Cerenkov counting: ~40% normal levels of ADH1 RNA were seen in *S3034*, 15% in *S3034b* and essentially none in *S3034a* homozygotes. Thus, the amounts of polyadenylated ADH1 RNA produced by the progenitor and mutant alleles reflect relative enzymatic activities in the induced roots.

Decreased levels of ADH1 RNA in the mutants are not due to a general failure of anaerobic metabolism. Seedlings homozygous for either the mutant alleles described here or any of several *Adh1*-null alleles (which have no ADH1 polypeptides) continue to support protein synthesis for over 12 h. Indeed, several null mutants produce normal levels of ADH1 RNA for

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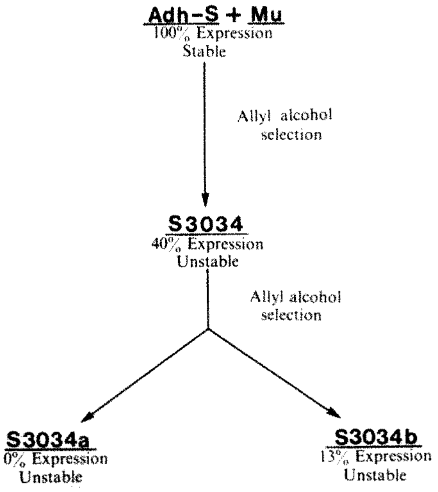

Fig. 1 The origin of our four *Adh1* alleles and a summary of their characteristics[9]. The *S* parent carried an electrophoretically identifiable EMS-induced mutant of *Adh2* which was recovered in *S3034* progeny, as was the somatic variegation phenotype characteristic of mutator lines. We have no lines capable of producing the *S3034* phenotype due to contamination. The *S* allele is defined as stable and 100% expressed. ADH1 activity was measured in mature scutella, in primary roots subjected to 24 h of anaerobic stress, and in pollen grains. Stable denotes (1) a phenotypic pollen revertant frequency similar to that of EMS-induced mutants at *Adh1-S*, that is, 2×10^{-6} ADH^+ pollen per total pollen[16]; and (2) a forward frequency of 2×10^{-7} ADH^- viable pollen per total viable pollen[17]. Reversion frequencies of mutant alleles are $1\text{–}2\times10^{-4}$ ADH^+ pollen per total pollen. Cytochemical staining of pollen or of germinating pollen *in situ* for ADH activity generated the data used to measure genetic stability.

that period (M. Sachs, personal communication and S.H., data not shown). Further, cDNA probes for other anaerobically induced RNA species detect the same levels of these other RNA species in all four genotypes (S.H., data not shown). We conclude that our four *Adh1* alleles differ in their quantitative expression because their ADH1 RNA levels differ, and that the observed changes are not merely secondary effects.

Figure 3 depicts Southern blots of genomic DNA probed with ADH1 cDNA from the plasmids pZmL84 and pKM1 (see Fig. 4 for descriptions of probes). Three conclusions can be drawn from the results. First, our hybridization conditions allow the cDNA to recognize a unique sequence of maize DNA. Second, in several cases *S3034* and its derivatives reside on fragments ~1.5 kb longer than fragments containing the progenitor allele. Third, despite the variations in ADH1 RNA levels, there are no detectable differences in endonuclease recognition sites around *S3034*, *S3034a* and *S3034b*.

A variety of single- and double-enzyme digests of genomic DNA allowed construction of the maps given in Fig. 4. Assignment of transcriptional polarity was simplified by the presence of a *Hin*dIII site in the *S* structural gene. Because the cDNA of pZmL84 has been sequenced[12] and correlated with peptide sequences (Inglis and Peacock, personal communication, and Kelly and M.F., unpublished data), there was no ambiguity in the assignment of polarity. The *Hin*dIII site in the recombinant clones allowed us to hybridize Southern blots of *Hin*dIII-digested DNA with 5′- and 3′-specific probes. Figure 4 insert illustrates the results of such an experiment. Clearly, the mutant *S3034* and its derivatives contain ~1.5 kb of additional DNA. This DNA element, containing a *Bst*EII recognition site, lies upstream from the ADH1-coding sequence represented in a probe which is 90% full-length (pKM1).

Although we have not proved that the *Mu* genetic background caused the DNA insertion at *Adh1*, evidence supports this hypothesis. Robertson's *Mu* background was crossed into the genotype from which the *S3034* pollen was selected. The pollen parent of *S3034* exhibited the behaviour characteristic of a *Mu* background[7], and the progeny exhibited the somatic variegation phenotype typical of *Mu* and heritable as a dominant non-mendelian trait (M.F. and J.N.S., to be published elsewhere). Furthermore, two additional *Mu*-induced mutants of *S* have been recovered, and they too contain 1.5 kb inserted DNA (R. Johns and J.N.S., unpublished data). Therefore, it seems most unlikely that the insertion of DNA at *S* occurred independently of *Mu*. However, we cannot conclude that the inserted sequences are Robertson's *Mu*. A molecular probe will be required to define *Mu* itself.

Evidence suggests that the quantitative regulation of *Adh1* by inserted DNA sequences occurs at the level of transcription or processing. The length of mature ADH1 RNA is unchanged, and the polypeptide produced by the mutants is unchanged in length, charge and specific activity[9]. We cannot exclude the possibility that less ADH1 RNA is seen in the mutants because it is more rapidly turned over, but our data are most compatible with altered transcription or processing.

The presence of inserted elements in *S3034a* and *S3034b* is not surprising, because these derivatives remain genetically unstable (Fig. 1). The lack of detectable differences between *S3034* and its derivatives was unexpected: transposition-associated events mapped at the DNA sequence level in other organisms involve conspicuous changes[13–15]. Although independent events occurring beyond the 20-kb window provided by our Southern blots might be responsible for decreases in expression of *S3034a* and *S3034b*, the distance required and the severity of the effect render this explanation unlikely. Alternatively, the unstable derivatives may differ from *S3034* by minor sequence changes that are below our threshold of detectability.

A possible interpretation of our results is that a *Mu* insertion has taken over some aspect of the regulation of *Adh1* expression. *Adh1-S* is expressed only in certain tissues; it is inducible by anaerobic stress and by the plant growth hormone, 2,4-dichlorophenoxyacetic acid[6]. Furthermore, we know that DNA acting in *cis* to *S* affects quantitative expression in an

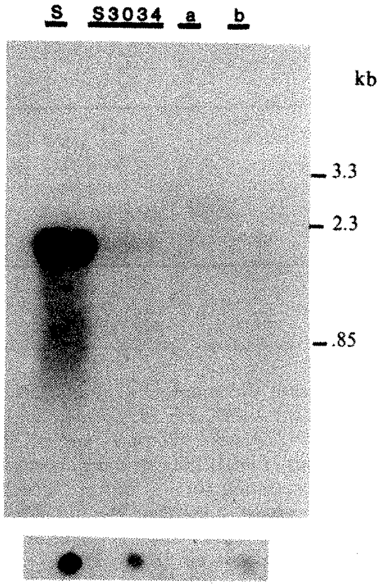

Fig. 2 Autoradiograms of Northern blots of polyadenylated RNA probed with the ADH1 cDNA plasmid pZmL84 (ref. 12) (see Fig. 4 legend for description of probes). Maize kernels of defined *Adh1* genotype were germinated and seedlings grown until primary roots were 5–10 cm long, at which time the seedlings were immersed in weakly buffered water for 12 h. Total RNA was isolated by the procedure of Lizardi and Engelberg[18]. We define polyadenylated RNA as that portion of total RNA recovered after selective binding to oligo(dT)-cellulose (T-2; Collaborative Research). The gel illustrated contained glyoxylated RNA (5 μg per track), separated on an agarose gel[19] and transferred to DPT paper by the unpublished procedure of B. Seed. Hybridizations were as described elsewhere[19] at 42 °C for 24 h with the addition of 100 $\mu g\ ml^{-1}$ polyadenosine; washes were as described by Gerlach *et al.*[12]. From left to right, polyadenylated RNA from *S*, *S3034*, *S3034a* (*a*) and *S3034b* (*b*) homozygotes.

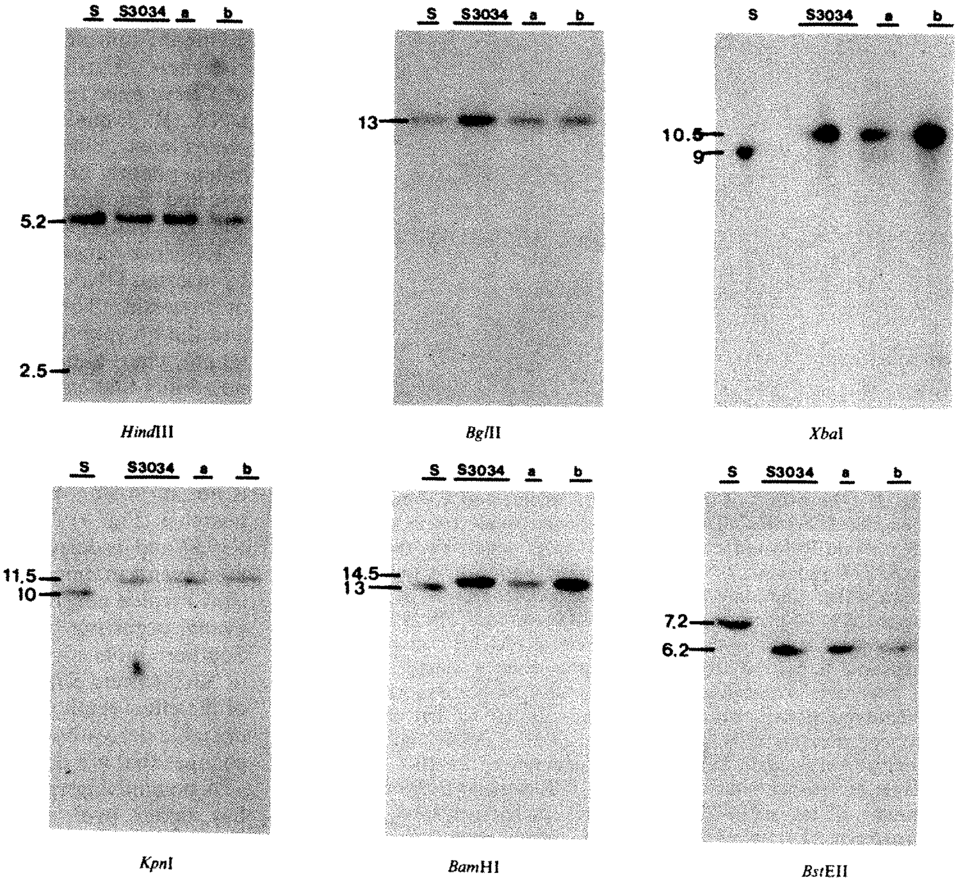

Fig. 3 Autoradiograms of probed Southern blots comparing the progenitor *S* allele with mutants *S3034*, *S3034a* and *S3034b*, and revealing the presence of ~1.5 kb additional DNA beyond the region of probe homology. Tracks are grouped by the endonuclease used to digest the genomic DNA before electrophoresis; *S*, *S3034a* and *S3034b* are displayed from left to right within each set. Genomic DNA was prepared by the method of Murray and Thompson[20], digested with restriction endonucleases as recommended by the supplier (BRL) and fractionated with 15 μg per track on 0.7% agarose (Seakem) gels in 90 mM Tris-base, 90 mM H_3BO_3, 3 mM EDTA, *p*H 8.3. Plasmid DNA was prepared by CsCl banding of cleared-lysate DNA[21] which had been precipitated with polyethylene glycol, and inserted cDNA sequences used as probes were isolated electrophoretically from plasmid endonuclease digests. Nick-translations, Southern blots and hybridizations were done using the procedure of Engel and Dodgson[22] with washing in 0.2×SSC, 0.1% SDS at 68 °C.

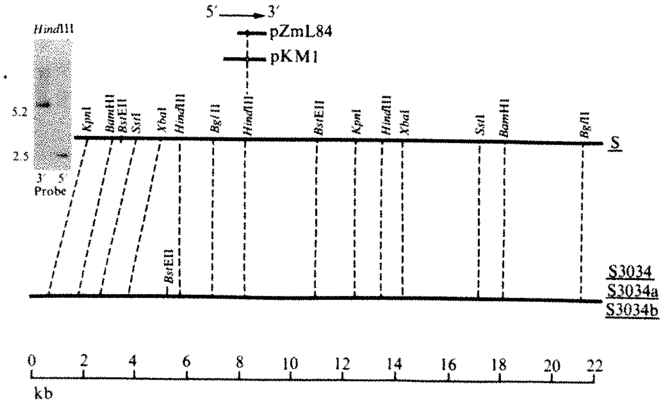

Fig. 4 Endonuclease site maps of genomic DNA containing *S* (top) and the mutant alleles *S3034*, *S3034a* and *S3034b* (bottom). The two cDNA probes, pZmL84 and pKM1, are aligned by their homologous *Hin*dIII sites, and the shortest possible transcriptional unit accounting for a 1,650-nucleotide transcript is represented by the arrow at the top. Plasmid pZmL84 was given by Gerlach *et al.*[12] and represents the 3′ half of ADH1 mRNA. Plasmid pKM1 was prepared from cDNA to anaerobically-induced mRNA, cloned in pBR322, detected as an anaerobically-stimulated sequence, and identified as ADH1 cDNA by hybridization to pZmL84; it contains ~1,500 base pairs (bp) of coding sequence. Localization of each endonuclease digestion site was confirmed with at least two sets of two-enzyme digests. Maps of *S3034*, *S3034a* and *S3034b* were generated independently. The inset illustrates the Southern blot of an *S Hin*dIII digest used to determine transcriptional orientation of the gene. The cDNA from pZmL84, with ~200 bp 5′ and 700 bp 3′ to the internal *Hin*dIII site, hybridized primarily to the large (5.2 kb) genomic fragment produced by *Hin*dIII digestion of *S*, while excised pKM1 sequences representing a region 5′ to the *Hin*dIII site recognized only the short (2.5 kb) genomic fragment. Note that the *Bst*EII site seen 5′ to the hybridizing sequences in *Adh1-S* is presumed present in *S3034* and its derivatives but cannot be detected due to the appearance of a new *Bst*EII site closer to the region of probe homology.

organ-specific way[9]. *S3034* and *S3034b* continue to exhibit all these aspects of normal regulation, producing 40% and 13%, respectively, of the level of ADH1 synthesized by *S*; organ specificity, developmental timing and inducibility remain unaltered. If *Mu* has assumed a regulatory function in the control of *Adh1-S* expression, it would seem to be a simple quantitative function that has been usurped.

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Antibodies to Z-DNA interact with form V DNA

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Annealing of single-stranded (ss) DNA rings of complementary base sequence gives rise to the so-called form V DNA which is characterized by a linking number of zero[1]. The similarity of the circular dichroism spectrum of form V DNA[1] with that of the 1:1 mixture of the low salt (B-type) and the high salt (Z-type) form of poly(dG-dC)·poly(dG-dC)[2,3] suggests that form V DNA might contain left-handed double-helical DNA of the Z-type. The discovery that the left-handed Z-form of poly(dG-dC)·poly(dG-dC) induces a strong immune response[4,5] has provided a novel means of searching for DNA of this particular conformation[6]. We therefore used antisera and monoclonal antibodies to Z-DNA to determine whether form V DNA contains stretches of left-handed double-helical conformation. We report here that form V DNA competes in the binding of antibodies to Z-form poly(dG-dC)·poly(dG-dC). The competition by form V DNA is similar to that of $(dG\text{-}dC)_5 \cdot (dG\text{-}dC)_5$. Thus, DNA of natural base sequence, when forced to adopt left-handed conformations by topological restrictions, is recognized by antibodies against Z-DNA.

Form V DNA, an 'unlinked' covalently closed, circular double-stranded (ds) molecule, is a form of DNA in which any right-handed double-helical structure has to be compensated by negative supercoils and left-handed duplex turns. It adopts a tightly folded structure, as revealed by sedimentation, electron microscopy and gel electrophoresis[1]. To date, it is the only form of DNA having a natural base sequence that displays a negative circular dichroism at 290 nm (Fig. 1). Such a negative circular dichroism band has been observed previously for poly(dG-dC)·poly(dG-dC) at high salt or ethanol concentration[2,3] and is characteristic of the left-handed double-helical conformation of Z-DNA[7].

Antisera against Z-DNA were obtained from a New Zealand White rabbit and BALB/c mice, respectively, after immunization with brominated poly(dG-dC)·poly(dG-dC), as described by Lafer *et al.*[4]. Such a mouse was used also for the selection of hybridomas producing monoclonal antibodies against Z-DNA (R.T., M. Leptin and F.M.P., in preparation). As the preparation of labelled form V DNA is very difficult, the interaction with antibodies was studied by the displacement of radioactively labelled poly(dG-dC)·poly(dG-dC); Fig. 2 summarizes the results of radioimmuno-competition assays in conditions in which poly(dG-dC)·poly(dG-dC) adopts the left-handed Z-form[2,7].

In the experimentally accessible range of concentrations, form V DNA of plasmid pBRβG2.17 competed with poly(dG-dC)·poly(dG-dC), whereas no competition was observed for open circular or covalently closed circular DNA with a natural superhelical density. No competition with circular ssDNA from phage M13 was found. This observation using rabbit antiserum has been substantiated with a purified monoclonal antibody against Z-DNA (Fig. 2*a*).

The concentration of form V DNA necessary for 50% inhibition of binding of the labelled polymer was about 400 times higher than obtained with unlabelled poly(dG-dC)·poly(dG-dC); this may be due either to much weaker binding of the antibodies to this DNA and/or to a low amount of Z-like DNA in form V. Figure 2*b* shows that the competition by oligo(dG-dC)·oligo(dG-dC) with a chain length of 10–14 base pairs (bp) is very similar to that of form V DNA. Poly d(I-5BrC)·poly d(I-5BrC), which also gives an inversion of the circular dichroism spectrum in high salt concentrations[8], competes even less and its effect is similar to that of $(dG\text{-}dC)_4 \cdot (dG\text{-}dC)_4$. The considerably stronger interaction with the polymeric antigen may be due to additional stabilization of the complex by the second binding site on the antibody compared with the short oligonucleotides, which apparently act as haptens.

To determine whether form V DNA interacts with antibodies at lower salt concentrations, the following more direct, qualitative approach was chosen (see Fig. 3): form V DNA was incubated with different amounts of antibodies to Z-DNA in 0.2 M NaCl, the reaction mixture applied to an agarose gel, and the DNA visualized by the fluorescence of bound ethidium bromide. The antibody–DNA complex does not enter the gel or is considerably retarded. Comparable results were obtained also with form V DNA prepared from phage PM2 (data not shown). The small amount of open circular DNA served as an internal standard, as it does not interact with the antiserum. For this type of assay, the final purification of form V DNA is unnecessary, as the antibodies discriminate between underwound closed circular DNA and the other forms of DNA.

The total amount of antibody necessary to retard 50% of the form V DNA could be estimated only very roughly and seems to be about half that necessary to precipitate the equivalent amount of poly(dG-dC)·poly(dG-dC) in 4 M NaCl. These results indicate that a considerable part of form V DNA interacts with antibodies to Z-DNA. The sequence of the plasmid pBRβG2.17 contains as alternating dG-dC stretches, one sequence of six base pairs and seven of five base pairs. If one considers also alternating purine–pyrimidine stretches, the lon-

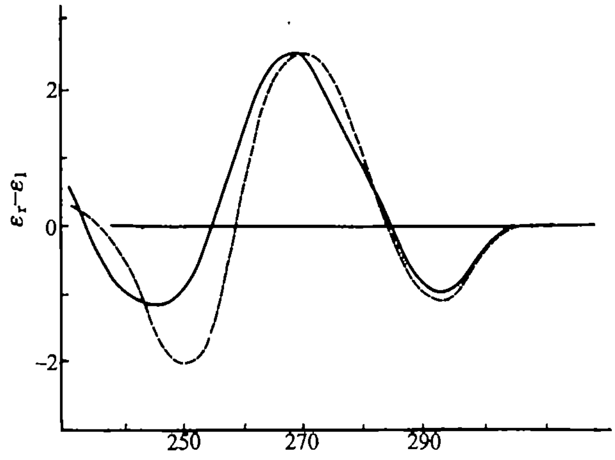

Fig. 1 Circular dichroism (CD) spectrum of form V DNA of pBRβG2.17 in 10 mM Tris-HCl *p*H 7.9, 1 mM EDTA (——) and a mixture of 50% low salt form (B-type) and 50% high salt form (Z-type) of poly(dG-dC)·poly(dG-dC) in 2.3 M NaCl, 10 mM Tris-HCl *p*H 7.9, 1 mM EDTA (– – –) as measured in a Cary 60 spectropolarimeter with CD attachment. The concentration of DNA was determined from the absorbance at 260 nm, using 6.8 $mM^{-1}\,cm^{-1}$ for form V DNA and 7.2 $mM^{-1}\,cm^{-1}$ for the low salt form of poly(dG-dC)·poly(dG-dC)[2]. Form V DNA was prepared by nicking closed circular DNA of the plasmid pBRβG2.17 with DNase I. Circular ssDNA was enriched by alkaline sucrose gradient centrifugation, brought to *p*H 9.0 and precipitated with ethanol. It was further purified by neutral sucrose gradient centrifugation as described by Stettler *et al.*[1]. We estimate that more than 95% of the DNA is form V, as judged in gel electrophoresis. Electron microscopy revealed highly folded molecules. Form V DNA could be cleaved with restriction endonuclease *Cfo*I and the fluorescence in 2.5 μM ethidium bromide *p*H 8.0 was almost the same before and after digestion, in contrast to naturally supercoiled form I DNA. $\varepsilon_r - \varepsilon_l$, difference of extinction coefficients for right and left circular polarized light ($M^{-1}\,cm^{-1}$).

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gest is 10 bp (Ph. Bucher, personal communication). Thus, it is tempting to conclude that a left-handed DNA conformation is not restricted to such a type of sequence as deduced from the X-ray structure determination of oligo(dC-dG)·oligo(dC-dG)[9,10]. This was the conclusion also of a study of the optical properties of form V DNA (S. Brahms *et al.*, in preparation).

Whether the apparently weaker interaction of our antisera with form V DNA than with the Z-form of poly(dG-dC)·poly(dG-dC) is due to subtle differences in the conformation of left-handed 'natural' DNA compared with that of poly(dG-dC)·poly(dG-dC), or to the lack of cooperation between the two binding sites on an antibody molecule, to structural perturbations at junctions between right- and left-handed segments[11], or to some other reason is unknown at present.

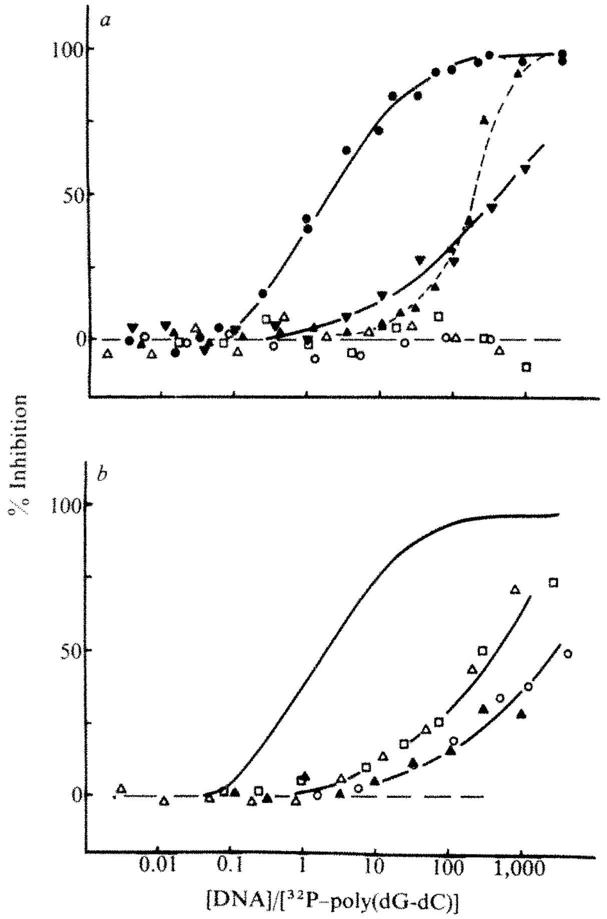

Fig. 2 Radioimmuno-competition assay with ^{32}P-labelled poly(dG-dC)·poly(dG-dC) in 4 M NaCl. *a*, Inhibition of the binding of radioactive poly(dG-dC)·poly(dG-dC) to rabbit antiserum by unlabelled poly(dG-dC)·poly(dG-dC) (●), and form V DNA (▲), prepared from the plasmid pBRβG2.17 (4,961 bp) according to Stettler *et al.*[1]. No inhibition is found with open circular (○) and superhelical, closed circular DNA (△) from the same plasmid, or with ssDNA prepared from phage M13 (□). ▼, A purified monoclonal antibody, Z-D11, and form V DNA were used in otherwise identical conditions. *b*, Inhibition by $(\text{dG-dC})_n \cdot (\text{dG-dC})_n$ of different chain lengths (○, 8; □, 10; △, 14 bp) prepared as described previously[12], and poly(dG-dC)·poly(dG-dC) (——) (see *a*); ▲, inhibition by poly d(I-5BrC)·poly d(I-5BrC) (PL Biochemicals). Poly(dG-dC)·poly(dG-dC) was synthesized as described previously[2] and the polymer was end-labelled with [α-^{32}P]dCTP (3,000 Ci mmol^{-1}) and DNA polymerase I from *Escherichia coli* to >5,000 c.p.m. ng^{-1}. Serum against Z-DNA was obtained from a rabbit immunized with brominated poly(dG-dC)·poly(dG-dC) according to Lafer *et al.*[4]. A given amount of DNA was incubated with 3.5–5 ng ^{32}P-labelled poly(dG-dC)·poly(dG-dC) and 0.05–0.07 µl rabbit antiserum or 14 ng purified monoclonal antibody in 100 µl solution (4 M NaCl, 60 mM sodium phosphate, 30 mM EDTA *p*H 8.0) for 1 h at room temperature. Goat antiserum to rabbit or mouse immunoglobulins was added, the mixture kept for another hour at room temperature, and then centrifuged. The radioactivity of the supernatant and washed pellet, respectively, was determined by Cherenkov counting. In the absence of competing DNA 30–60%, and using serum from a non-immunized rabbit, less than 0.5% of the radioactivity was found in the pellet.

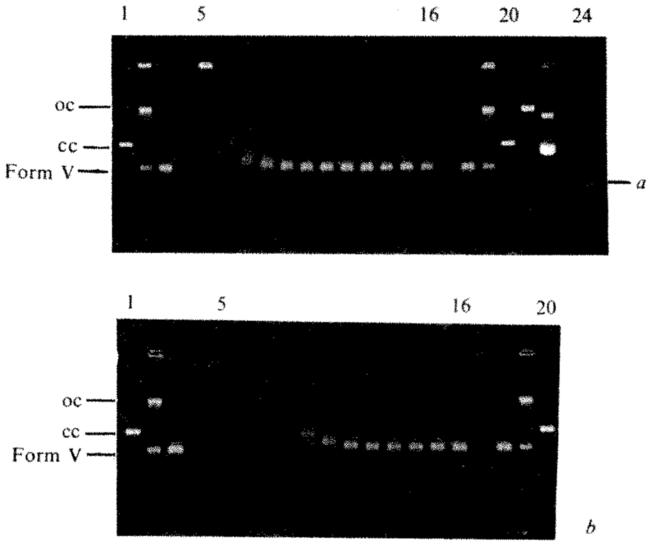

Fig. 3 Electrophoresis of form V DNA of plasmid pBRβG2.17 after reaction with different amounts of antibodies to Z-DNA in serial 1:2 dilution (lanes 5–16): starting with *a*, 1.3 µl rabbit antiserum; *b*, 2 µg monoclonal antibody Z-D11. Form V DNA (115 ng) was incubated for 1 h at room temperature with the indicated amount of antibody in a total volume of 10 µl (0.2 M NaCl, 60 mM sodium phosphate, 30 mM EDTA *p*H 8.0), 2.5 µl 50% glycerol added, and the solution loaded on to a 1% agarose gel. The electrophoresis buffer contained 36 mM Tris, 30 mM NaH_2PO_4, 1 mM EDTA, 1.25 µM ethidium bromide. Electrophoresis was at 5 V cm^{-1} for ~2 h and the gels were photographed under UV illumination. Control sera showed no effect and the following DNA samples were included as references: lanes 1,20, natural closed circular (cc) DNA; lanes 2,19, form V DNA before neutral sucrose gradient centrifugation with open circular (oc) and 'unlinked' closed circular DNA; lanes 3,18, form V DNA without antibody; 21, open circular DNA; 22, pBR322 DNA; 24, form V DNA denatured in 0.2 M NaOH.

We thank Professor Theo Koller for encouragement and helpful discussions, the Schweizer Nationalfonds zur Förderung der wissenschaftlichen Forschung (Professor Th. Koller), and the Deutsche Forschungsgemeinschaft (Po 155/6) for financial support.

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Corrigendum

In the letter 'Redesigning enzyme structure by site-directed mutagenesis: tyrosyl tRNA synthetase and ATP binding' by G. Winter *et al.*, *Nature* **299,** 756–758 (1982), 'lower K_m' in the last line of the introductory paragraph should read 'poorer K_m'.

CHRISTMAS BOOKS SUPPLEMENT

Where do rainbows come from?

Peter Newmark looks at some of the science books for children recently published in Britain.*

WHAT an odd and lopsided view of science children would be left with from this selection of books. For the younger age groups the emphasis is on natural history and dinosaurs with a modicum of computers and a sprinkling of the four elements. For children approaching the end of their first decade the tables are turned: computers and other artefacts of high technology take first place from natural history and fossils, with some elementary physics thrown in. But, for whatever the age, of physiology, chemistry and genetics there is seldom a word. Even within the limited range of topics that seem to be considered palatable to the customer there are many missed opportunities for slipping in a scientific concept or two, surreptitiously if needs be.

Under-6s

It is a pity, for example, that Althea Braithwaite did not introduce the concept of photosynthesis — but not the word — in **Tree** (£2.95), one of Longman's *Life Cycle Books.* The tree in question, an oak, is said to obtain food and water through its roots, and to take back food from the leaves before they fall. When on the tree the role of dead leaves is, we are told, to protect new buds, and when off it to protect fallen acorns; but the living leaves are never given any function. Equally it would not have been impossible to have hinted at the concept of inheritance or of territory in **Foxes** (£2.95), another of the same series. That apart, both books are blessed with bright and uncluttered illustrations and — how nice to see them — delightful endplates.

Much more sophisticated illustrations adorn Vanessa Luff's **The River** (A&C Black, £3.95), 12 double-page spreads depicting the course of a river from its moorland source to the sea. Each spread is of a different habitat with representative fauna and flora, and carries only a nominal sentence or two of text. There is, however, an identification key to each picture at the back of the book. *The River* is for parent and child to "read" together with much adult pleasure to be had from the illustrations. There is rather less in the way of adult entertainment in either **Frogs** or **Butterflies** (Heinemann/Quixote Press, £2.95 each; to be published in the United States by Putnam at $6.95), two rather crudely illustrated Natural Pop-Ups. However the pop-up pictures and other paper mechanics work quite well and whisk one through the life-cycle of the beast concerned in all of five double pages. Your infant prodigy might notice that frogs mate before the female produces eggs whereas the female butterfly appears to go in for virgin birth.

6–8-year-olds

So, too, does — or rather did — the brontosaur, if I am not being misled by no less an authority than Beverly Halstead. My impression is gained from **A Brontosaur: The Life Story Unearthed** (Collins, £3.95), illustrated by Jenny Halstead and written by Beverly. In its 15 double-page spreads is recounted the 120-year life of a brontosaur named Ajax, whose mate lays eggs every spring as though that were the sole meaning of coming into season. The story is nicely illustrated and well told in about 100 words per page. Cleverly, there is also a boxed sentence or two per page which explains the evidence on which the depicted scene has been reconstructed.

Passing quickly over **Space Mission** and **Underwater Mission** by Terry Pastor (Methuen–Walker, £3.95 each), pop-up books that are more gimmick than value, brings me to four topic books from the same publishing stable: two of five in the *Animal Lives* series and the first two of *The Elements.* The animal lives are those of **The Spider** and **The Fox** (£3.95 each; published in the United States by Dial, $9.95 each). Both books are written by Margaret Lane, biographer of Beatrix Potter. Those whose sympathy is with the uneatable rather than the unspeakable in the fox hunt should be warned that Margaret Lane's views of the chase are that "It is not an easy sport, for the fox has his own methods of escape"; and anyone with a predisposition to arachnophobia had better be spared Barbara Firth's larger-than-life illustrations in *The Spider.* Both her illustrations and those by Kenneth Lilly for *The Fox* are stunning. David Lloyd's **Air** (£3.95) — one of *The Elements* books — is also well illustrated with a text that is informative but dull; in **Fire**, by John Satchwell (£3.95), the text outdoes the illustrations. All four books are well worth giving to the child who has started to investigate and write about particular topics at school — although sod's law being what it is, the chosen topic is bound never to coincide with the books available.

A much more unusual and altogether inspired book, the first in the *Tell Me About. . .* series, is **The Earth and the Sky** (Collins, £3.50) which has been translated from a French text by Pierre Avérous. It takes the form of about 75 questions and answers, three to the double page, on the weather, the Earth and the sky. Not only do they help the child to understand but also the adult to explain how, for example, night comes, where rainbows come from and why one cannot see that the Earth is round. Clever and inventive answers are given in both words and illustrations; the book is a Godsend for the harassed parent.

8–11-year-olds

There is also an inspired and out of the ordinary book for older children in Peter Ward's **The Adventures of Charles Darwin** (Cambridge University Press; £3.75, $7.95). George Carter, a cabin-boy on the *Beagle*, befriends Mr Darwin who proceeds to share his thoughts on such matters as slavery, the presence of sea shells on mountain tops, the variety of finches on the Galapagos Islands and much else besides. The educative role is never obtrusive, being carefully placed among adventures with South American gauchos and under Argentinian gunfire. Finely illustrated, and with chapter headings such as "Mr Darwin gets Ants in his Pants", this is writing for children at its best.

Dinosaurs by David Lambert (Kingfisher/Crown; £5.95, $6.95) is a much more conventional book but excellent in its own way. Its 90 large pages have vivid illustrations, graphics and photographs which provide a good counter-balance to the rather academic text. Complete with glossary and index, large-lizard-lovers will find this a nice book for browsing and better still for reference.

For reference sake, perhaps to coincide with a holiday, Penny Anderson's **The**

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*Where possible, details of availability and price in the United States are given. All readers should check prices before ordering.

Wildlife of Mountains and Moorlands (Macdonald, £3.95) — one of a series of six *Nature in Focus* books — can also be recommended. Sixteen well-illustrated double-page spreads cover the designated habitat, its fauna and flora and its changes with the seasons. Useful for the purposes of identification, the scientific titbits are a welcome bonus. Almost at the other end of the scale is **Animals of the Mountains and Forests** (Burke; £4.95, $12.95) and its five companion volumes in the series *Animals and their Environment*. The choice of topics to occupy the 32 double pages appears to be far too arbitrary and the whole approach rather incoherent. Why, for instance, devote two whole pages to the seven-spot ladybird and include several species that are as typical of suburbia as of the habitat of the title? A redeeming feature of the books is their use of impressive colour photographs wherever possible.

Inanimate in subject but not in style is **Earthquakes and Volcanoes** by Sara Steel (A&C Black, £3.95), one of this publisher's Junior Reference Books. It starts with 10 pages on the causes of earthquakes and volcanoes and then devotes 50 more to descriptions of some of the most famous, from Santorini to Mount St Helens. Full of dramatic photographs and gory detail, this will be a sure-fire success as a topic book. It is more of a puzzle to know what to make of **Sam's System: A Guide to Computers** (Dent, £3.95), in which Rosemary Court adopts the anthropomorphic approach: ". . . it had two little feet dangling over the edge of the desk, two hands folded neatly under the screen . . . . I'm TAK the terminal. TAK's my pet name — but I have other names too. VDU and CRT, for instance". By chatting to TAK the terminal, Sid Software, Susie the Supermarket Stock Control Program and a host of others, Sam (of the title) becomes wise beyond his years. I could not stomach this book, but buy it if you are intent upon training a tiny technocrat.

Darwin agogo on Tierra del Fuego, together with George Carter the *Beagle's* cabin-boy. (From *The Adventures of Charles Darwin*, reviewed here.)

A much more conventional approach is taken by Gareth Renowden and Nigel Hawkes in **Video** and **Space Shuttle**, respectively (Collins/Gloucester Press; £2.95, $9.40), in the *Inside Story* series. Each has 30 large pages, with illustrations taking more space than text. There are diagrams, cut-aways, a little technical data, a glossary and an index but in the end too much knowledge is assumed and too little information provided. Nevertheless these are attractive books of some value.

For something completely different your child might like to learn that 'flu viruses from outer space can result in the spread of epidemics on Earth. It's Fred Hoyle again but here in combination with his son Geoffrey and with the decency for the sake of children to wrap his proposals firmly in the cloth of science fiction. **The Planet of Death** is one of four of their science fiction books, published by Ladybird at 60 pence ($1.95) apiece, and all good yarns. *The Planet of Death* is typical: William, a good schoolboy, has considerably less time for the vivacious Kiryl than for her father, Professor Gamma, who is adept at the art of dematerialization when it is necessary for our three heroes to visit another planet.

11-year-olds and over

There are both books of facts and books of experiments for this age group, the former including three *Granada Guides* on **Fossils, Computers** and **Robots** (£1.95 each). Pocket-sized, with glossary, index and many illustrations, children will find them nice enough to dip into but not especially good as serious reference books. More on computers is to be found in the 40 double-page, large-format **Discovering Computers** which, with **Discovering the Weather,** is the latest in Longman's *Discovering* series (£6.95 each; Little, Brown, $12.95 each). The writing is dull and the approach dry but the child already interested in either subject would be happy to be given them.

Far less routine and largely successful in their intent are numbers 9 to 12 in the series *How We Found Out About . . .* by Isaac Asimov, also from Longman (£2.75 each). Published in the 1970s in America by Walker, these four new volumes deal with **Energy, Germs, Antarctica** and **Dinosaurs** (what, no computers?), largely from a historical point of view. *Germs,* for example, starts with the first microscope and ends with the discovery of viruses. The usual childish angles are largely eschewed — there is almost nothing on the death of dinosaurs or solar energy — but a whole chapter on entropy. Not surprisingly, Asimov carries it off with ease.

Thorough assessment of the five books of experiments would have taken more time and children than were available. In the case of **How to Make Square Eggs** by Paul Temple and Ralph Levinson (Beaver, £0.95), the title experiment worked but I cannot guarantee equal success for the square tomato (start with a seed and encase an infant fruit in a box) or for the experiment entitled "A plant that eats animals" (first buy a Venus fly trap). A paperback, with 40 experiments rated by difficulty and some aimed for the classroom rather than home, it would engender both amusement and frustration. Leonard Marsh's **The Guinness Book for Young Scientists** (GBR Educational, £2.95) is better in that most of the experiments use household materials, although very basic chemistry apparatus is required for some and a trip to the chemist for others. Experiments include how to make plastic from milk and hydrogen sulphide from a metal bottle cap!

Like the other books from Methuen-Walker, **Amazing Air** and **Supermotion** (£3.95 each), two of the *Science Club* series, are beautifully designed and illustrated. There are about 30 experiments in each book, carefully explained and illustrated, step by step, with attention to detail, supplies and safety. *Amazing Air* has experiments on such phenomena as air pressure, oxidation and electrolysis; spinning, magnetism and balance are included in *Supermotion*. Morrow will publish the books in the United States in spring 1983, price $5.25.

Last, but very definitely not least, is **The Science Book** by Sara Stein, a book first published in the United States and now available in Britain (Heinemann/Workman; £4.95, $6.95). It is full of humour, zany facts (why don't cats beg for cookies? — because they have no taste receptors for sugar), and experiments to attract and educate children. To learn about kidney function, eat asparagus and follow your nose. Get to grips with the genome by cloning a plant (unfortunately for British readers, here the recommended species are all American) and with anatomy by checking out the chicken bones on a Sunday roast.

The Science Book is not simply a list of experiments. Rather it is an unorthodox primer, with unorthodox demonstrations, on much that is of interest to a growing child. There are sections on charges (electrical and magnetic), protists and plants. But the bulk of the book is concerned with physiology while chemistry and genetics get a reasonable look in. This is the only book of the whole batch to be centred on such sciences; for that and its refreshing approach, I give thanks and can forgive some errors of judgement. □

Peter Newmark, the father of a six-, a nine- and a twelve-year-old, is Deputy Editor of Nature.

Risibility research reviewed

William H. Press

More Random Walks in Science. Compiled by Robert L. Weber. Pp.207. ISBN 0-85498-040-7. (The Institute of Physics/Heyden: 1982.) £9.95, $19.50.

WE TRY to keep it a secret, but we all know that research and teaching are not what science is all about. Rather those doubtless worthy activities are pale diversions from the real business of science, namely the invention, dissemination and elaboration of funny anecdotes about colleagues, parodies, doggerel and the occasional pithy aphorism or *bon mot.*

A Random Walk in Science, which was published about a decade ago as a collection of such fundamental material, has come to be held as a kind of *urtext* for all science, the ultimate peak of that pyramid which begins with primary publications at the bottom and then ascends to review articles, reviews of reviews and so on.

Now this sequel, collecting some 175 or so additional short pieces, can be added to that lofty edifice. Here, for everyone, there is something, whether whimsy like "The Use of Small Dogs in Physics Teaching" or broad jokes like the investigator who ate dehydrated food for 28 days and then gained 108 lbs in ten minutes while caught in a rainstorm. Parody ranges from the predictably amusing "I am the very model of . . .", to the arcane, such as Shelley's *Ozymandias* ("Two vast and trunkless legs of stone/Stand in the desert . . . Look on my works, ye Mighty, and despair!. . .") rewritten as the geology paper "Twin Limb-like Basalt Columns and Their Relationship to Plate Tectonics".

Scientists cannot resist applying scientific observation to their own endeavours, yielding, for example, a description of their university hierarchy, from the Dean who leaps tall buildings in a single bound, through Professor (leaps short buildings with a running start and favourable winds), graduate student (runs into buildings) and finally department secretary (lifts buildings and walks under them). There is also a piece which rings true on the various types of obstructionists on committees and panels, and another on the "game" of refereeing. (The author's goal is to publish a worthless paper; the referee's is to have a major contribution to the field refused. "No matter what degree of rigor the author uses, the referee replies by saying that it is not the correct one.").

Anecdotes are nicely represented. We have the famous theoretician Sir Harold Jeffreys, consultant to an oil company, listening attentively to a full day's presentation and then offering his (only) advice: "I'm glad it's your problem and not mine". We have Bessel receiving from King Friedrich Wilhelm not the needed funds to improve his observatory, but rather a medal for having attained such excellent results with primitive equipment. We have Pauli in heaven, hearing with mounting impatience God's explanation of the proton–electron mass ratio and finally snapping back, "Wrong!". And Rutherford's memorable encounter in a glass-teapot works.

Literary hoax is represented by the paper coauthored by a cat (so that the sole human author could use the word "we" over an editor's objections), and by the biography of Claude Litre, the celebrated eighteenth-century maker of wine bottles after whom the volumetric unit is (putatively) named.

Courtesy Cambridge Medical Instruments Ltd.

From *More Random Walks in Science*.

Having fun in the laboratory? This doleful character appears to have doubts about the safety of the original electrocardiograph (*c.* 1911).

The volume also contains perhaps the finest scientific biography of Sir William Rowan Hamilton ever penned, here quoted in its entirety:

Higgledy, piggledy
William R. Hamilton
Wrote on mechanics, in-
Frequently joked,

Wrote on quaternions,
Tackled intractable
Hydrodynamical
Problems, and croaked.

While perhaps unsuitable as an introductory text, this volume will certainly fill the needs of advanced students and practitioners in the field. □

William H. Press is Professor of Astronomy and Physics, and Chairman of the Department of Astronomy, at Harvard University.

How to be a scientist

Walter Gratzer

Winning the Games Scientists Play. By Carl J. Sindermann. Pp.285. ISBN 0-306-41075-3. (Plenum: 1982.) $15.95, £10.05.

WE ALL agree that there is more to science than doing research. The politics of professions in which scope for the exercise of power is limited or illusory, such as the academic or the ecclesiastical, tend to be conducted with particular ferocity and vindictiveness, redeemed only by the incompetence of most of the protagonists. It is a subject that invites satirical dissection: someone should do for our profession what A.P. Herbert did for (or to) the law.

I picked up Dr Sindermann's book with eager anticipation. The title is full of promise and from the dust-jacket the author, wearing a natty check and the suggestion of a knowing smile, looks at one appraisingly with gimlet eyes — clearly A Man that Waiters Spring to Serve. I averted my gaze and turned to page 1. But what was this? The first sentence averred that the book had been written for "thousands of serious dedicated scientists", such as myself, "to ensure that they [may] participate fully and joyously in a genuinely wonderful occupation". If this was a manual of gamesmanship for the scientist it evidently owed little to Stephen Potter and all too much, if not to Lord Chesterfield then certainly to Dale Carnegie: how to win patrons and influence the Federation of American Biochemists.

There is, it is true, a sprinkling of anecdotes and many a genial aside, but there is not much levity, for Dr Sindermann's purpose is serious. His thesis is that for most scientists, the pleasures of the laboratory bench are in the long, or even the short run inadequate; and that if they are to enjoy a full and rewarding career they have no option but to throw themselves into the parascientific activities that beckon on all sides, such as attending and preferably organizing meetings, sitting on committees and running international commissions. He would evidently reject the proposition (first enunciated, I believe, by no less an authority than Professor Milton Friedman) that useless work drives out useful work. In short, while insisting that there is no substitute for prowess at research, Dr Sindermann urges his readers to join that travelling circus of Flying Dutchmen, whose chief and urgent preoccupation it is to keep as far away from their laboratories for as much of the year as possible, and leave the research in the calloused hands of their postdocs, technicians and PhD students.

As a guide to social and professional advancement in such company, the book is, so far as I can judge, excellent. Its most insistent message is that one must cultivate an uncompromisingly professional approach to all the activities in question — including even the successful pursuit of your quarry at the conference cocktail party. Dr Sindermann dispenses much good advice on how to give a paper at a meeting, how to make effective use of slides and how to deal with discussion. He descants on the best way to run a session and on the problems of organizing the whole affair yourself. Anxious novices will no longer have to discover the hard way where to go for financial sponsorship, how to cope with recalcitrant and temperamental foreigners and what to watch out for in drawing up an agreement with a commercial conference organizing company. Dr Sindermann writes here with an authority that can only stem from long experience and careful study.

On the more general aspects of the *arriviste's* upward trajectory, however, such as the craft of committee chairmanship (or in the Newspeak favoured by Dr Sindermann, chairpersonship), he has much less to say that is not a mite obvious. He does not offer such helpful hints in Advanced Chairmanship Technique as that once imparted to me by an expert in the field: ensure that the room is overheated and make large volumes of tea and coffee available, so that the ensuing discomfort will inhibit prolonged argument when eventually you introduce the issue of real importance. Again, it is related that Maurice Bowra, a university politician of transcendent virtuosity, would overturn a ten to one majority against him by observing that an *impasse* seemed to have been reached, and perhaps therefore the meeting should be adjourned. Dr Sindermann's advice in his "chairman's creed" is more along the lines that a chairman must be firm but not domineering. Most of what he has to say might be more succinctly summed up in Senator Dirksen's well-known adage that the oil can is mightier than the sword.

In the later chapters there is a good deal of padding, and to my mind not much that is helpful when it comes to coping with the "midlife crisis" or with such alien forces as probing politicians from the outside world and women in the laboratory. It is curious that a book which encompasses such a wide range of opportunities and pitfalls — and even advises you how to deport yourself in the laboratory when in the throes of lust — makes not a mention of teaching. We are meant to conclude presumably that this is an occupation that plays no part in the process of getting ahead.

Dr Sindermann is an Establishment man. His advice is that you order your life according to Establishment rules, and then you too may become known for being noted. He has no patience with the unsuccessful and mediocre, who, he holds, are simply not trying: you should show them no mercy. His book may well help to reclaim some of these fallen, and should give encouragement to the walking wounded in the battle for academic survival. But where it may comfort the afflicted, it will certainly not afflict the comfortable. There are, it seems to me, more useful maxims for young scientists, struggling to prevail in a hostile climate, than any that Dr Sindermann has to offer. One that seems to me to encapsulate an enduring truth comes from Alexander Cohen's *Journal of Irreproducible Results* and goes like this: if a research project is not worth doing at all, it is not worth doing properly. Cap that, Dr Sindermann. ☐

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Now you solve it, now you don't . . .

David Jones

Aha! Gotcha: Paradoxes to Puzzle and Delight. By Martin Gardner. Pp.164. Hbk ISBN 0-7167-1414-0; pbk ISBN 0-7167-1361-0. (W.H. Freeman: 1982.) Hbk $15.95, £11.20; pbk $8.95, £5.60.

MARTIN Gardner is probably best known for his feature in *Scientific American*, "Mathematical Games", which ran regularly from 1956 to 1981; but he has also published many other articles and books, both light-hearted and serious, on mathematical topics. *Aha! Gotcha* is definitely light-hearted, but still true to its subject, and similar in format and spirit to its companion-volume *Aha! Insight.*

Both books contain about 70 self-contained entries, each exploring some intriguing mathematical trick, puzzle or theorem. *Aha! Gotcha* deals with paradoxes, pointing out the role they play in sharpening mathematical reasoning, and presenting them in six sub-divisions: logic, number, geometry, probability, statistics and time (this last strays over the boundary of mathematics into physics).

Here are discussed the familiar paradoxes of self-referring statements (the lying Cretan), infinite sums and curves (the Snowflake), probabilistic fallacies (Pascal's wager) and the Zeno paradoxes (Achilles and the tortoise). Here also are many more modern and subtle paradoxes: the Unexpected Tiger, Newcomb's paradox of the perfect thought-reader, the voting paradoxes which subvert our hopes for a perfect democracy.

Each entry provides a simple but well-reasoned discussion of the chosen paradox, and some examples — one of them in strip-cartoon format. These cartoons are characteristic of the *Aha!* books; they are highly stylized drawings by Jim Glen, and feature characters such as Dr Zeta, the extraterrestrial scientist, and Mr Randi, the world-famous magician. I can imagine them either delighting or infuriating the reader. Intellectually, they provide a sort of pictorial commentary on the text which makes its reasoning more accessible, and gives the book a cheerful and whimsical atmosphere.

Aha! Gotcha should appeal to students and non-specialist readers who would be discouraged by a more formal presentation; at the same time even professional mathematicians may be glad to see their subject sketched in this ingenious way. Many of the paradoxes are not yet resolved — does the occurrence of a purple *cow* really provide support for the assertion that all crows are black? For those who wish to pursue such questions further, the book has a useful reference-list of further reading. Less dedicated readers will merely enjoy puzzling over the paradoxes and springing them on their friends. ☐

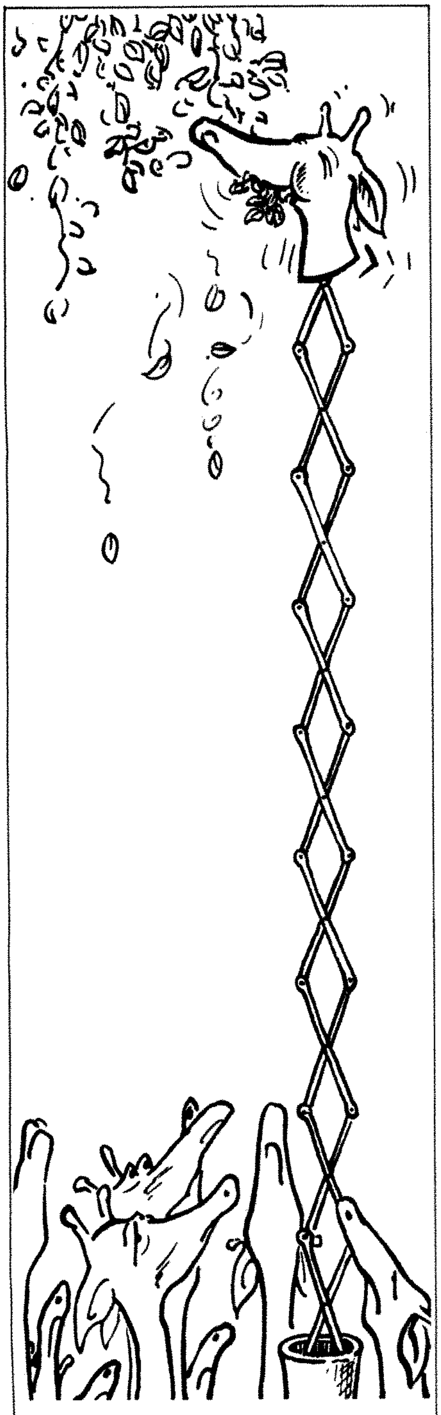
David Jones is a Guest Member of Staff with the Physical Chemistry Department of the University of Newcastle upon Tyne. His book, The Inventions of Daedalus, *derived from his weekly column in* New Scientist, *has just been published by W.H. Freeman.*

Darwin and evolution: the comic cut

J.S. Jones

Darwin for Beginners. By Jonathan Miller and Borin van Loon. Pp.176. Hbk ISBN 0-906495-95-4; pbk ISBN 0-906495-96-2. (Writers and Readers/W.W. Norton/Pantheon: 1982.) Hbk £7.95, $11.95; pbk £2.95, $3.95.

EINSTEIN, Marx, food, Freud, capitalism — and Darwin. These are some of the titles in a series of illustrated books "for beginners" which discuss complex subjects in a simple way using a mixture of cartoons and text. How does Charles Darwin emerge from his treatment as the thinking man's Andy Capp?



Stretching a point about evolutionary theory. One of Borin van Loon's illustrations in *Darwin for Beginners*.

The short answer is: remarkably well. Jonathan Miller gives us a concise and clear account of Darwinism, which is complemented by some two hundred crude but powerful illustrations by Borin van Loon. Darwin's predecessors, his life and the *Origin of Species* are covered in a body of text enough to provide only the preface for most books on evolution. We learn not only of the well-known elements of our hero's life, but also of neglected episodes such as Captain Fitzroy's early distrust of Darwin because the shape of his nose betrayed signs of laziness and hesitancy. The laziness — which provoked his father to exclaim that "You care for nothing but shooting, dogs and rat-catching. You will be a disgrace to yourself and all your family" — disappeared early, but the hesitancy remained for the rest of his days.

Darwin's lack of confidence and his hypochondria are often blamed on the effects of Chagas' disease (which he may have contracted in South America). Here a dramatic illustration shows him cowering before an infected mosquito — which is unfortunate as the vector is a wingless bloodsucking bug. There are many quotations from Darwin's writings, published and unpublished, including his musings on the advantages of a "nice soft wife on a sofa"; advantages which include, according to the accompanying cartoon, sharing a bed with Freud and Marx.

The treatment of Darwin's predecessors and his own work is outstandingly clear. We hear of Cuvier, to whom evolution was a process of stability interrupted by miraculous change, and of Paley, who saw in the perfection of living things the proof of an intelligent designer. That of neo-Darwinism and the "modern synthesis" is less successful, and shows signs of confusion. There is the compulsory nod towards neo-Cuvierism — or evolution by jerks as it is known to its critics — in which mysterious forces lead to evolutionary equilibria punctuated by rapid change, but nothing on neo-Paleyism, the view that all animal structure and behaviour is of necessity perfectly adaptive and is hence always a proof of strong natural selection.

Nevertheless, the book as a whole is a success. It can be recommended to biologists who have ceased to be entertained by evolution as well as to newcomers to the subject. Darwin, Doonesbury, Dennis the Menace: in this post-literate society to be immortalized in a comic strip is the ultimate accolade payable to a great mind. What next — a cartoon version of *Principia Mathematica*? ☐

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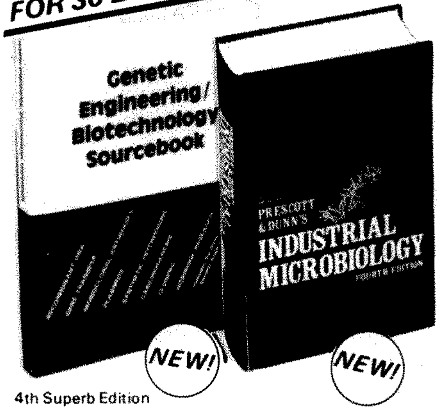

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Knocking about in the nineteen-eighties

Bernard Dixon

The Cult of the Expert. By Brian J. Ford. Pp.194. ISBN 0-241-10476-9. (Hamish Hamilton: 1982.) £5.95.

ONE of Dwight D. Eisenhower's more memorable remarks was that "public policy could itself become the captive of a scientific–technological elite". That forecast was underscored some years later by Dr Herbert York, Defense Department adviser to both Eisenhower and Kennedy, when he warned that the possible decision to initiate nuclear annihilation was passing "from the hands of statesmen to lower-level officers and ultimately computing machines and the technicians who program them".

Clearly, this is a theme, with parallels in fields as disparate as health and nutrition, communications and education, which is worthy of serious attention. Yet it has not been widely discussed — certainly not in books. Theodore Roszak's *The Making of a Counter Culture* (Faber & Faber, 1970) touched on the problem, but was more concerned with the domination of our society by the products and ideas of science than with the role of experts *per se*. So there certainly is an opportunity for a keen examination of specialists, their habits and attitudes, and their effect on the rest of us — citizens, consumers, customers, captives.

The Cult of the Expert appears at first sight to be such a book. It is (to take his conclusion first) Mr Ford's fleshing out of the following thesis:

> From the way you are born to the manner of your dying, great and earth-shattering decisions are taken behind closed doors by people who may well have closed minds. For the Expert all that matters is that they are seen to have a monopoly on power. Their long and meaningless words, elaborate and expensive apparatus stacked in high security buildings, the machines that they secretly know no more than anyone else how to use for the good of the people, are all part of today's power structure. The omnipotence of the Expert pronouncement and the unchallengeable right to spend huge sums of money gratifying a lust that evolved only to be gratified; these are the pattern for the future.

In support of these somewhat inelegant propositions, Mr Ford tells us at great length that experts employ contorted language. He provides many examples such as "mobility transportation system" for "car", quotes a passage we may have missed from *Private Eye* showing that the word "situation" has become comic, and uses a menu to compare "plain language" with French. He states that Ulcerative Dermal Necrosis ("as far as it means anything at all") really means that ulcers of dead skin form on the surface of fish, and he devotes an entire chapter to rules such as "never use one word where many will do".

Mr Ford recounts the banning of cyclamates, the N-ray story, the polywater affair, the Kammerer and Burt episodes, and the work of Stanley Milgram, to show science in a variety of bad lights. He says that an Expert could ban air, and explains that nitrogen and its other constituents can be dangerous. He criticizes new maths and decimalization (predicting that Experts will next want to divide the year into 100 parts). He then complains about bank cards and gives many examples of "the way in which the infectious enthusiasm for believing in computers has come close to harming our lives", before vouchsafing that there are innumerable other such examples. He gives pupils rules such as "Do just what the teacher says, and on no account argue" and opines that the purpose of a university is "to provide a closetted and confined period of indoctrination for malleable minds". Finally, with embarrassing innocence of the real world, Mr Ford tells us that "elaborate and expensive research institutes" are needed only for Experts to perpetuate themselves, and that libraries will go on paying inflated prices for journals.

Anyone setting out to survey such (some may think familiar) territory should decide whether their book is to be humorous or serious, and whether the aim is to flatter readers or make them feel uneasy. Mr Ford has done neither. He begins by taking a swipe at his readers' poor education and calling them "ordinary people", but then expects them to join in his sarcastic superiority. He enjoys his own knockabout fun but sours it all by being bitchy. He gets serious things wrong (the butter–margarine controversy was about the contribution of their respective fatty acids to coronary heart disease, not about which was more fattening) while trying to be witty by assuring us that Experts are above the law. And his publishers do not help by producing what they (presumably) intended to be a rib-tickling book with a dust jacket highlighting the author's "acid pen". Curious.

Mr Ford is not, I understand, a qualified scientist. So his words come with considerably less clout than those of, say, Sir Peter Medawar when he gently mocks some of his fellow experts. But then the very idea of Medawar writing ***this*** book is much funnier than anything Mr Ford has to say. □

Bernard Dixon, formerly Editor of New Scientist *and European Editor of* Omni, *is a science writer and consultant.*

Pictures, prose and a world of vegetation

Philip Stott

Green Planet: The Story of Plant Life on Earth. Edited by David M. Moore. Pp.288. ISBN 0-521-24610-5. (Cambridge University Press: 1982.) £12.50, $27.50.

THE many recent attempts of science publishers to blend the visually attractive with the academically acceptable have not always proved felicitous. Solid content has all too often yielded to the simply picturesque. One notable exception is Duvigneaud's splendidly gallic *La Synthèse Écologique* (Doin, Paris; 1974). This new encyclopaedia of plant ecology and geography is another.

There are two clear reasons for the book's success. First, the text, which has been skilfully compiled from the separate contributions of 30 specialists, dominates the book and is, in the main, both comprehensive and accurate. Secondly, the visual material is used to illustrate and exemplify the text rather than, as is so often the case, the words being mere adjuncts to pretty pictures. In addition, it is especially gratifying to welcome a general work on biogeography which acknowledges nearly all of the main traditions of the subject. In the seven well-constructed chapters, the encyclopaedia thus embraces the key aspects of ecology, of plant geography in its classical sense — meaning the study of the spatial distribution of plant taxa over the surface of the Earth — and of the role of man in nature. This last theme is perhaps the least satisfactorily covered and I was sorry to see no mention of the important applied textbooks of I.G. Simmons in the bibliography at the end of the book.

Obviously, in a survey of such breadth, there will be some blemishes. The term "ecology", for example, was not coined by the German biologist, Ernst Haeckel, in 1869 (p.21), but appears as early as 1858 in a letter of the American essayist and nature-lover, Henry David Thoreau; moreover, Haeckel himself first used the term in 1866. It is pleasing, however, to see both autecology and synecology adequately discussed.

Cladistics is well explained, as one would expect, by Heywood in his precise and useful entry on taxonomy and systematics, but nowhere is the possible phytogeographical significance of cladistic relationships explored. The closely related topic of vicariance biogeography is largely ignored, save for a short passage on vicariance written in a more traditional vein. It is also regrettable that many of the plant and vegetation distributions are still mapped on non-equal-area projections, which unacceptably exaggerate the northern latitudes, whilst the large and well-chosen glossary is occasionally somewhat opaque in its definitions.

But these are all quibbles. This encyclopaedia is both elegant and informative. The editor and his contributors are to be congratulated on providing what is really a colourful and modern Polunin (*Introduction to Plant Geography and Some Related Sciences*, published by Longman in 1960). It should prove of enormous value to both sixth formers and undergraduates, as well as to a wider readership of naturalists and botanists. ☐

Philip Stott is a Lecturer in Biogeography at the School of Oriental and African Studies, University of London, and author of Historical Plant Geography *(George Allen & Unwin, 1981).*

Other men's flowers, other men's gardens

Sandra Raphael

The Illustration of Plants & Gardens 1500–1850. By Vera Kaden. Pp.106. Hbk ISBN 0-11-290375-4; pbk ISBN 0-11-290331-2. (HMSO/Kraus Thomson: 1982.) Hbk £12, $25; pbk £6.95, $14.
The Origins of Garden Plants. By John Fisher. Pp.338. ISBN 0-09-463760-1. (Constable: 1982.) £12.95.

VERA Kaden's collection of pictures taken from botanical and gardening books in the library of the Victoria and Albert Museum made a good first impression on me by using a beautiful Ehret drawing of a lily — one of my favourites — to adorn its dust jacket. The source of the illustrations selected indicates that the books and manuscripts were chosen for aesthetic appeal rather than purely botanical interest, but such a limitation does not, of course, exclude most of the greatest plant books; nor, indeed, does it detract from the book's contents.

A short introduction, sparingly decorated with black-and-white illustrations, canters briskly through a potted history of garden design, with excursions to provide necessary details of herbals and flower books and the processes by which they were illustrated. There is also a brief account of floral symbolism.

The remaining two-thirds of the book is made up of plates, about half of them in colour, all with descriptive captions. The arrangement is more or less chronological, starting with the earliest woodcuts of plants from the late fifteenth century and ending with a couple of plates from Victorian albums of flower paintings. The plants and the gardens go hand in hand, with pictures to show changing tastes in design, from the enclosed mediaeval garden to architectural Italian ones, formal patterns from France and the English style of landscaping. Nearly all the colour plates, reasonably enough, are devoted to the work of the flower painters, with Maria Sibylla Merian, Ehret, Redouté and Franz Bauer representing the greatest of them. Altogether the book makes an attractive guide for those taking their first steps in garden history.

From The Illustration of Plants & Gardens 1500–1850

John Fisher's book, *The Origins of Garden Plants*, also offers background information to the curious gardener, in this case on the subject of plant-hunting and the introduction of some of the botanical immigrants now comfortably settled in the British Isles. The book starts with a couple of chapters on the native plants of Britain before going on to those cultivated for medicinal and other purposes in mediaeval times. Then we are launched into the search for decorative plants, which very often ran parallel with the exploration of new colonies. Almost every part of the world has been visited by amateur or professional plant-hunters, with obvious consequences in the range of plants now available to us — the rhododendron belt of the Home Counties would not have been possible without Hooker's Indian journeys and the Chinese collections of several French and British explorers; and several of the species of conifer covering all too many of our hills are the descendants of the North American discoveries of David Douglas and his contemporaries.

Collectors in Europe began with attempts to identify the plants described by classical writers. The seventeenth century brought the first great wave of introductions from the New World, many (including *Tradescantia virginiana*) via the gardens of the Tradescants, father and son. The French in Canada and the southern part of the continent sent back plants to Paris, the Spaniards in South America to Madrid. French missionary priests, particularly in China, sent home plants as well as the first report of the giant panda.

In the late eighteenth and early nineteenth century, Sir Joseph Banks, First as a collector, later as the impresario of other men's travels, commanded the field. His patronage, allied with that of the Royal and Horticultural Societies and Kew Gardens — all three institutions firmly under Banks's wing — sent professional collectors across the globe; South Africa, Australia, China and Japan were all areas in which he was interested. Later other patrons or nurserymen financed major expeditions, a pattern that lasted to relatively recent times and the journeys of Ludlow and Sherriff and of Kingdon-Ward. Now the flood of new plants has slackened a little and efforts are being made to prevent the loss of older species driven into neglect by the vagaries of horticultural fashion.

The subject of Mr Fisher's book must appeal to any gardener with the slightest interest in the history of the plants he or she grows. The text, however, is not always accurate; nor are the captions to the illustrations. No one reading the book would guess that the Chelsea Physic Garden still survives, although its future seems once again to be in jeopardy. And it is said that Robert Brown, his Australian travels over, was "afterwards librarian of the Linnean Society". He did not stay in that position, though, for he was Banks's botanical heir, an eminent scientist in his own right and eventually President of the Linnean too.

From The Illustration of Plants & Gardens 1500–1850

The author notes of John Bartram, an eighteenth-century Quaker naturalist living in Pennsylvania, "His spelling remained consistently unreliable". A similar warning is needed here, for Mr Fisher's book appears to have escaped both copy-editing and proof-reading. Spelling in English and Latin is equally eccentric: even dates are often wrong. Let the buyer enjoy this pleasant book, but also beware of trusting the accuracy of the details. ☐

Sandra Raphael is Senior Editor (Natural History) in the Dictionary Department of Oxford University Press. She is currently working on a new edition of Wilfrid Blunt's The Art of Botanical Illustration.

Bees for science and bees for keeping

John Powell

Bees and Mankind. By John B. Free. Pp.155. ISBN 0-04-638001-9. (George Allen & Unwin: 1982.) £9.50, $17.95.

DR JOHN Free is internationally acknowledged as an authority on bees and pollination; his published work in the scientific literature is extensive. Any book from such a source is therefore bound to be looked upon as of some importance in beekeeping circles.

It is interesting to note that of Dr Free's books to date, half have been written for children and it is evident that he has mastered the art of expressing a complex subject in a straightforward and lucid manner. In confirmation of this, *Bees and Mankind* is a fascinating and easily readable work, though its structure is unusual. The book is split into three parts entitled "Bees: Solitary and Social", "Honeybees" and "Beekeeping: Past and Present". The reader, however, is more likely to regard the text as divided into two.

Parts I and II together provide a brief account of two selected solitary bees, *Osmia rufa* and *Megachile rotundata*, and a more detailed treatment of bumblebees and honeybees. Each chapter is broken into short sections, each headed by a quotation from such divers sources as Virgil and J.G. Whittier. It is the sharp contrast between the cool text of Dr Free and the colour of some of the poetry that provides much of the charm of this book, though I found it took some time to get used to it. This then is not a book to dip into — at least not at first acquaintance.

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There is a distinct change of style when we enter Part III, effectively the second half of the book, and move from the strictly factual material to a narrative of man's relationship with bees over 9,000 years, from primitive honey-hunting to the sophisticated mechanization of the late twentieth century. This must be one of the most complete accounts available and John Free's wide experience of the bee species involved is evident in the way he has been able to make a coherent whole from the often fragmentary evidence. There is, too, more than an interesting story here; in their unobtrusive way bees have had a profound influence on man's social, religious and cultural development.

Like the bees themselves, the sting of the book comes in the tail. The closing pages deal with the influence and availability of pollinating insects on agricultural production, both in intensively farmed areas and in developing countries.

The book is liberally illustrated and is beautifully set-out and produced. Although the subject matter is vast there is throughout an element of speculation which binds the whole together, be it the socialization of bees or man's manipulation of that social organization. It would be a pity if *Bees and Mankind* remained solely within beekeeping circles; it could be read profitably by all who are involved in conservation and planning, agricultural development, international aid programmes and anthropology. ☐

John Powell, a research physicist, is author of The World of a Beehive *(Faber & Faber, 1979).*

High in Africa

Margaret Sharman

Kilimanjaro. By John Reader. Pp.84. ISBN 0-241-10683-4. (Elm Tree, London/Universe: 1982.) £12.95, $25.

KILIMANJARO is not only Africa's highest mountain, and so a magnet for climbers, it is also an ecologist's delight. Within a few miles one finds tropical forest, alpine flora, near-desert, glaciation and the world's biggest volcano. Such variety provides scope for the excellent photographs, ranging from majestic landscapes to haunting close-ups, with which John Reader adorns his record of one man's pilgrimage to Kilimanjaro.

One usually sees the mountain for the first time as a backdrop for the migrating herds of the Serengeti Plain, the elephants of Tsavo, or even, on a clear day, the ex-colonial suburbs of Nairobi, over 100 miles away. It rises from the burning heat of the thorn savannah, and it is no wonder that the reports of missionaries Krapf and Rebmann in the mid-nineteenth century were rejected as the imaginings of fevered brains and poor eyesight.

Yet tales of snow-capped mountains in Africa were not new. As an introduction to the highly personal account of his first climb, the author traces the story through the centuries, as Europeans overcame the obstacles — adverse geographical conditions, slave traders and the spears of tribesmen defending their homeland against white men bent on "discovering" Africa. (I am dismayed, incidentally, to find that the kink in the Kenya–Tanzania border is not the result of Queen Victoria giving Kaiser Bill the present to end all presents.) African legends, too, there are in plenty; and tales of those who were intrepid enough to brave the "fiery beings" and the fingers that "died", to obtain samples of "the silver-like stuff [which] when brought down in bottles, proved to be nothing but water".

Climbing the mountain is one of the things Westerners who live in East Africa aim to do "one day". It is everybody's mountain in that, while it is climbable without expertise, the twin peaks of Kibo and Mawenzi provide severe challenges, some of which are here described with concomitant gruesome tales of misadventure.

The snows of Kilimanjaro are melting rapidly (the ice has been in retreat since *c.*1700), and the melting process is responsible for the fantastic ice edifices which are among the most striking images in the book. Lower down in tropical splendour are the giants — the lobelias, and the groundsels which grow up to four metres high and may be 100 years old. How plants withstand temperature extremes and at the same time cope with intense ultraviolet radiation and the threat of being exhumed by solifluction is explained in the last section of the book.

Near the mountain's summit are found cryptozoic spiders, and crystallized butterflies which have been blown up by the "anti-trade wind" — a wind which explains the conventional picture of a snow-white summit riding high on a cloud layer and apparently completely detached from the earth below. This is the one photograph which every tourist takes home, even if only on a postcard, and which John Reader omits. But with 82 colour plates one cannot cavil at that. Here is an attractive and very readable book.

Margaret Sharman is a freelance editor specializing in books on African history.

Half and half in the national parks

Brian Bertram

A Field Guide to the National Parks of East Africa. By John G. Williams and Norman Arlott. Pp.336. ISBN 0-00-219215-2. (Collins/Stephen Greene Press: 1982.) £7.95, $29.95.

LIKE ALL Gaul, this handy field guide is divided into three parts. The first part surveys the national parks, game reserves and other faunal areas of Kenya, Tanzania and Uganda, the second is a guide to the larger mammals and the third a guide to the commoner birds of East Africa.

Unlike all Gaul, the standard of the three parts varies widely. The 70 pages on the mammals are well-presented and accurate; they are in effect the same as in the original 1967 version of this field guide. With the help of 13 pages of illustrations, they enable the visitor to identify almost any mammal he is likely to encounter. The 92 pages on the commoner birds are also good, and are the completely new part of the book. They replace the section in the previous edition which dealt with the rarer birds, and instead provide a condensed version of its companion volume on the birds one is more likely to see. Fifteen pages of effective colour illustrations help in the identification.

But as for the remaining part, "Oh dear" was my reaction. The opening 160 pages on the national parks and other wildlife areas are absolutely riddled with mistakes, and are going to mislead a great many people. Partly this is because they are hopelessly out of date, and have not been changed from the previous edition. Thus, to pick out just a couple of examples, the area of Meru National Park is now less than half the area John Williams states, and the "motor boat available for anyone wishing to explore the country from the rivers" was actually burnt to ashes at least a dozen years ago.

Other errors abound on the maps, caused partly by some careless re-drawing of long out-of-date maps. Thus roads (in Tsavo) and boundaries (in Amboseli) are often in the wrong place, and lodges are omitted (in Serengeti) or misplaced (in Tsavo). Even whole reserves are misplaced — one is shown over 200 km away from where it actually is. Advice on routes is often wrong and details in the species lists for the parks are faulty; for example the abundant hippo is omitted from Tsavo (despite a successful film about them there), and Patas monkeys have not been seen in Meru by anyone who has worked there for long. And so on.

This is very sad, because there is a wealth of knowledgeable people in East Africa who could have weeded out almost all of these mistakes, and made the book far more useful. As it is, one has to say "Don't trust the first half of it". But there is no other comparable guide, so if you are going to East Africa you had better get hold of it, warts and all. And take with it a pinch of salt and some proper maps. □

Brian Bertram is Curator of Mammals at The Zoological Society of London.

Private lives

John R. Krebs

The Discovery of Animal Behaviour. By John Sparks. Pp.288. ISBN 0-00-219061-3. (Collins/Little, Brown: 1982.) £12.95, $24.95.

FOLLOWING along the now well-used trail opened up by *Life on Earth, The Discovery of Animal Behaviour* is the book of the television series recently shown on BBC2. The text follows almost verbatim the script of the programmes, and although well written it loses some of its impact when not accompanied by the film. But pictures there are in plenty; every second page is a double-sided colour plate of high quality.

For those unfamiliar with the series, the book is essentially a history of ethology, highlighting the contributions and circumstances of key figures from the middle ages to the 1960s. The "stars" include Ray, White, Leroy, Darwin, Romanes, Spalding, Loeb, Watson, Skinner, Pavlov, Lorenz, Tinbergen and von Frisch, a fairly uncontroversial list. In spite of its title, the last two chapters of the book (covering the mid-twentieth century) are almost exclusively about ethology and sociobiology: psychologists fade out after Skinner and Harlow.

Those hoping to gasp at arcane marvels of animal behaviour or to be enlightened about the big issues in present-day ethological research may be disappointed, for this is as much an account of the people as of their subject. It is hardly gripping stuff, especially for those not familiar with animal behaviour; after all, most naturalists are men of quotidian habits.

One of the nice features of the book is the way in which the discoveries and attitudes of individuals are related to their personal circumstances. For many the stimulus and opportunity to study animal behaviour was linked with relaxing country residences — a mill by an Austrian lake, a rambling house close to the Danube, a country cottage at Dunskaith; in stark contrast, we are told that the theoretician W.D. Hamilton did much of his thinking while waiting at Waterloo Station!

Another recurrent theme is how easily scientists were led to generalize their findings to an unreasonable point. Loeb, the discoverer of phototaxis considered whether the human mind could be explained in terms of taxes and tropisms; Romanes asked whether spiders might appreciate music; and Schelderup-Ebbe, the man who discovered dominance hierarchies, subsumed the relationship between God and the Devil in his account of social organization. It is easy to smile, but less easy to see whether or not one has one's own musical spiders. □

John R. Krebs is Lecturer in Zoology at the Edward Grey Institute and E.P. Abraham Fellow of Pembroke College, University of Oxford.

Home on the range — wildebeest (gnu) in the Amboseli National Park, Kenya. The snow-capped Kilimanjaro rises in the background. (Photograph David Keith Jones.)

And the gentlemen went there two by two

Bernard Stonehouse

Voyage Through the Antarctic. By Richard Adams and Ronald Lockley. Pp.160. UK ISBN 0-7139-1396-7; US ISBN 0-394-52858-1. (Allen Lane/Knopf: 1982.) £8.95, $13.95. *Antarctic Wildlife.* By Eric Hosking and Bryan Sage. Pp.160. ISBN 0-7099-1215-3. (Croom Helm/Facts on File: 1982.) £12.95, $22.95.

ANTARCTICA and the Southern Ocean are among the few remaining bastions of wildlife, though with a long history of depredation at the hands of human beings. Southern fur seals were hunted almost to extinction on the peripheral islands in the eighteenth and nineteenth centuries, and whale stocks devastated in the present century. Elephant seals and even penguins were boiled by the thousand for the golden oil in their blubber. Now the killing of the larger animals has almost stopped; attention turns to harvesting the fish and krill that will form man's final bonanza from the Antarctic.

For many years scientists and their support teams had sole rights of travel in the far south. Today tour ships are regular visitors, loaded with enthusiastic passengers who saw Alaska last year, the Galapagos the year before, and are now doing Antarctica. Tourists on the hallowed ground where Scott, Amundsen and Byrd once stood? Yes, and good luck to them. Nowhere in the world is the scenery grander and less spoiled by human artefacts; nowhere do seals and seabirds gather in more spectacular concentrations; and nowhere are wild animals more readily approachable by nature-lovers with camera, notebook and wide-eyed wonder. The two books reviewed here arose from tours laid on by Lindblad Travel, one of the earliest operators to brave the fury of stormy seas, elitist scientists and anxious administrators to take ordinary people (well, ordinary *wealthy* people) to the Antarctic. As a result, thousands have seen the southern wonderland who would otherwise have been excluded, and there are correspondingly more who can write about it, read about it and, if necessary, root for it from first-hand knowledge.

Voyage Through the Antarctic, the smaller and cheaper volume, is the narrative of a journey from Tierra del Fuego to Stewart Island, New Zealand, via the South Shetland Islands, Antarctic Peninsula, Peter I Øy and the edge of the Pacific sector pack ice, calling at McMurdo Sound, skirting Cape Adare and the Balleny Islands, and visiting Macquarie and Campbell Islands on the homeward run. Two generations ago this voyage would have been regarded as hazardous in the extreme; even now it is a voyage that polar scientists (myself among them) would be glad to experience for the research opportunities offered. Yet this and other trips like it are the stock-in-trade of tourist operators these days. Richard Adams, naturalist, novelist (*Watership Down, Shardik, The Plague Dogs*) and RSPCA luminary tells the plain tale, which naturalist and writer Ronald Lockley (*Man against Nature, Orielton, Ocean Wanderers*) embellishes with italicized passages of concentrated natural history.

This may well have been a practical way (perhaps the only way) of combining the talents of two celebrated writers who live far apart and write in widely-diverse idioms. For me the device fails; the two voices sing in different keys. Both authors are self conscious, Adams, the jolly tourist, Lockley, lyrical–authoritative; both have written far better, and neither rises fully to what could and should have been a splendid occasion. Peter Hirst-Smith's photographs too are disappointing. The few pages of colour glow excellently, but the many black-and-white prints spread through the text could have been selected more rigorously and printed less muddily; the best are fair, and the worst have a potato-print quality that does them no justice. But this is a good adventure story, with a lot of information and a deal of enthusiasm for Antarctica and its animals that make the book worthwhile.

Antarctic Wildlife is for me a much more satisfactory book, larger and almost fifty per cent dearer, but a far better production all round. This is not the story of a voyage; it is an account — indeed a celebration — of Antarctic life, seen through the perceptive eye of Eric Hosking and discussed in scholarly but readable vein by Bryan Sage. The Old Master has lost none of his touch; these are superb photographs, printed to a high standard in both colour and black-and-white. I cavil only at a designer who on occasion lets the central gutter spoil the layouts unnecessarily. The seven chapters cover the historical background, continental, maritime and subantarctic ecology, and the life histories of penguins, albatrosses, other birds and seals; there is a final chapter on wildlife photography that many will find valuable. *Antarctic Wildlife* is an authoritative and beautiful volume, a book fit to match its subject. □

Bernard Stonehouse is at the Scott Polar Research Institute, University of Cambridge, and editor of Polar Record.

Poles apart

Wildlife in the Arctic, specifically the polar bear, is the subject of *Lords of the Arctic* by Richard C. Davids with photographs by Dan Guravich. The book is an account of scientific observations, conservation problems and the authors' experiences in their search for this elusive animal. Published by Macmillan, New York, *Lords of the Arctic* costs $29.95.

The natural history of mammals anew

I.J. Linn

Mammals in the British Isles. By L. Harrison Matthews. Pp.208. ISBN 0-00-219738-3. (Collins: 1982.) £10.95.

A NEW title from the prolific pen of Dr Harrison Matthews is always a significant event in the world of theriology; and this *is* a new title, not merely a new edition of his earlier book, *British Mammals,* in the same *New Naturalist* series.

As both author and publisher are at pains to point out, the tremendous flowering of theriological literature which has taken place in the past 20 years or so has enabled the author to dispense with much of the basic introductory material which was necessary in the older book, and to follow through a specific line of thought with simplicity and clarity. That line is the one which amateur naturalist and professional zoologist alike contemplate with wonder and delight, namely the extraordinary detail and perfection of the adaptations of animals, in this case British mammals, to the environments in which they live. It is the line of thought which has given us the academic disciplines of sociobiology and behavioural ecology, and it also generates many of the questions which thinking people of many differing ages and backgrounds ask of biologists — questions which are often very difficult to answer. This book will help all of these people.

Dr Matthews lays a firm foundation by spending most of the first third of the book reviewing the geological, climatic and human influences which have shaped the range of habitats available to British mammals. He then goes on to write lucidly and effectively about the various facets of ecology and behaviour which define a mammal's adaptivity, presenting hard scientific information and modern theory in a palatable and easily absorbed manner.

The book as a whole is spiced by Dr Matthews's trenchant and perceptive comments upon humanity, past and present. It is an excellent introduction to the natural history of British mammals for anyone, amateur or professional, who is interested in real animals in the real world.

Ian Linn is Senior Lecturer in Biological Sciences at the University of Exeter, and a founder member of the Mammal Society.

Birds from the kitchen window . . . and much further afield

John Andrews

THE KITCHEN window is the starting point for many people's interest in birds — an interest which they may never take beyond the front gate. **The Garden Bird Book,** edited by David Glue (Macmillan, London, £7.95), is right up their street. Compiled by the British Trust for Ornithology, its aim is to help people encourage birds into their lives by providing suitable food and shelter. It contains lots of practical advice applicable to the small suburban garden as much as to the fortunate minority whose spacious grounds attract woodpeckers and water rails. More than that, the text is thoroughly interesting, and packed with insights into the lives and survival strategies of common birds. Well-illustrated, this is certainly a book to enthuse the garden bird-lover and perhaps encourage forays further afield.

Anyone who does venture the transition from bird-lover into bird-watcher must soon attempt to choose from a plethora of field identification guides. Little has been added to the current choice by John Gooders and Terence Lambert's **British Birds** (Collins/Stephen Greene Press; £12.95, $39.95). On the one hand, Lambert's illustrations though aesthetically pleasing are of little help to field identification: indeed, as printed, some of the colour tones are positively misleading. On the other hand, John Gooders' text is largely field-identification material inappropriate in a book which might fit the parcel shelf but certainly not an anorak pocket. In style and format, the book is very reminiscent of the Readers Digest/AA *Book of British Birds* published in 1969 which remains the more readable and entertaining volume.

Field identification skills usually depend less on feather-perfect knowledge of plumage than on knowing where and how different species comport themselves — just as most people recognize acquaintances at a distance without any ability to describe them in detail. For the fairly inexpert, **Birdwatching on Inland Fresh Waters** by Malcolm Ogilvie (Severn House, £8.95) provides this kind of helpful information on identification and basic ecology for a group of habitats rich in birds and accordingly popular with birders. Opening with a description of Britain's freshwater habitats, the author, who is on the Wildfowl Trust staff, moves on to discuss many aspects of wetland bird adaptation and behaviour, and concludes by explaining some of the current research and conservation work directed at these birds.

Strangely, many bird-watchers seem happy to go through life without ever understanding how the objects of their identification skills actually function. For those who are moved to do so, a good and not overly technical account is provided in the book **How Birds Work** by Ron Freethy (Blandford/Sterling; £8.95, $15.95). The author has evidently reviewed the current literature to produce an up-to-date explanation of opinion on evolution, structure, flight, respiration, feeding, the nervous system, breeding, migration, behaviour and distribution. Unfortunately, a rag-bag of illustrations adds nothing to the book's appearance and little to its information.

One area of common ground between bird-lover, bird-watcher and the scientific ornithologist (pompous fellow!) is the almost universal appeal of birds of prey. In Britain, a peregrine falcon is perhaps *the* bird to see on a day in the field. Following the crash in peregrine populations due to organochlorines, work on captive breeding as a conservation technique was developed in the United States by Dr Tom Cade, Professor of Ornithology at Cornell. Now, in **Falcons of the World** (Collins/Cornell University Press; £15, $38.50), he summarizes present knowledge not only of peregrines but of the entire falcon genus. The first quarter of the book is a general review of falcon biology and ecology, the bulk of the text comprising essays on each of the 39 species with handsome plates by David Digby, whose double spread of a gyr falcon striking at a ptarmigan is stunning. Though a glossy, large-format work, this is not to be dismissed as a coffee table volume: it is a scholarly and appealing account of a group of birds which, as Cade says, "more than any other combines form and function in a perfection that can hold men in spellbound wonder".

Study of a heron, by Eric Ennion. The illustration is taken from *The Living Birds of Eric Ennion,* a collection of drawings and paintings which displays the late Dr Ennion's remarkable and varied talents as writer, ornithologist and artist. The book includes a commentary by John Busby and is published by Gollancz, price £9.95.

Though birders have become increasingly well-travelled in the past decade, most would be hard pressed to know where to look for cotingas. The family, which includes bell-birds, umbrella birds and the cock of the rock (ah! now you know) contains about 65 species, mostly specialized fruit eaters, from the forests of South and Central America. In **The Cotingas** (Oxford University Press/Cornell University Press; £30, $45) David Snow attempts to provide the first comprehensive account of the group, drawing on his own studies as well as on the work of others. Though hardly likely to appeal to a wide readership, the book is certainly of interest to those studying avian courtship behaviour and, as the author sadly remarks,

> if it stimulates further fieldwork and encourages wider interest in a family of birds that suddenly, in the last thirty years, after millions of years of evolution, are as threatened as the forests in which they have evolved, it will have achieved another of its purposes.

Closer to home, there is concern for a number of species which show recent declines. One is the barn owl and it is opportune that a major monograph has now appeared. **The Barn Owl** by D. Bunn, A. Warburton and R. Wilson (Poyser/Buteo; £12.60, $32.50), is based on the equivalent of 38 years of field work between 1963 and 1982, covering virtually all the habitat types used by the species in Britain. The authors' findings are compared with those of other workers world-wide — for the barn owl is perhaps the most widespread land bird in the world. A readable style, good presentation and illustration make the book a model of its type. Once again, ornithology owes a debt to the amateurs who "lose inordinate amounts of sleep . . . . get eaten alive by a varied assortment of biting flies . . . . and spend long hours when absolutely nothing happens".

It was amateurs too who undertook the three years' field work which produced in the mid-1970s the Register of Ornithological Sites. This ambitious project, run by the British Trust for Ornithology, sought to identify and evaluate as many as possible of the ornithologically important sites in the United Kingdom. Inevitably, it fell short of comprehensive coverage but it has provided conservation planners with a valuable aid for individual site protection, a yardstick and methodology for site evaluation, a pointer to habitat types or areas requiring further coverage and, last but not least, a basic evaluation of the overall resource now published as **Bird**

Habitats in Britain (Poyser/Buteo; £13, $35). The author, Rob Fuller, was responsible for organization of the national survey and he has drawn not only on its results but on many other sources to describe the composition of bird communities in different habitat types: he also comments on the pressures from current land-uses, notably agriculture and forestry which are the major modifiers of habitats and thus the greatest threat to a number of British species.

A mapping exercise of a different character is represented by Colin Harrison's **Atlas of the Birds of the Western Palaearctic** (Collins/Princeton University Press; £12.95, $25) which shows the summer, winter and resident distribution of all species normally occurring in Europe, North Africa and the Middle East. The text gives accounts of each species' status, climatic range, typical habitats and sites and there are pleasant, if rather irrelevant, half-tone drawings of the species concerned on each page. The layout and presentation are clear and the work provides a partial up-date of Voous' masterpiece, *Atlas of European Birds*, now 20 years old. Perhaps inevitably in such a complex exercise, there are mapping errors but, used with care, the book will be of value, not least to the select band of birders who seek to pursue their hobby wherever birds themselves go. □

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Africa's avifauna

C.M. Perrins

The Birds of Africa, Vol. 1. By Leslie H. Brown, Emil K. Urban and Kenneth Newman. Pp.536. ISBN 0-12-137301-0. (Academic: 1982.) £53.40, $99.

NO WORK covering the birds of the whole of Africa has been published for over 20 years. *The Birds of Africa* is intended to remedy this state of affairs, and when completed will comprise at least five volumes (with the possibility of a further one to include the birds of Malagasy and the Indian Ocean).

This, the first volume, deals with 10 orders of non-passerine birds from ostriches to birds of prey, some 270 species in all. It starts with 31 useful pages of general information on the geology, climate, geography and habitats of Africa, and with an introduction to the avifauna, the number of species involved and their migrations.

In the main part of the book the information given for each species is fairly standard handbook material, with details of range and status, description, field characters, voice, general and breeding habits and food, together with a distribution map and one or more pictures on the colour plates. On average some 1½ pages is allotted to each bird, though a few well-known species (such as the ostrich) get as many as five pages and a few of the vagrants only a few lines. Throughout the book the writing is clear and informative, and emphasizes field biology, especially ecological and behavioural studies, the latter often including line-drawings to amplify the text.

Although the standard of presentation is high, there are some shortcomings. For instance the distribution maps raise a few problems. Many in other handbooks show summer and winter ranges. This is more difficult when dealing with African birds because different areas experience such very different seasons. Ideally, however, it ought to be possible to depict breeding and non-breeding distributions and it is a little disappointing that this has not been done, except in a very few cases. In addition there is some inconsistency — albeit minor — in the treatment of distributions of the birds of offshore islands and in mapping bird distributions in Malagasy.

Bird books often stand or fall by the quality of their illustrations, but here the plates — by Peter Hayman and Martin Woodcock — are well up to standard. Some of the paintings are on the small side; in the extreme case there are over 70 birds on a single page. However on the whole they are acceptable because of the large format of the book, and as a group they succeed remarkably well in being both accurate and aesthetically pleasing. For most species there are at least two pictures showing the different sexes or summer and winter plumages; in addition there is often a painting of a young bird.

One minor worry concerned the amount of space available for each species. The dust-jacket states that there will be a further three volumes. However, this first volume includes only about 270 of the 1,850 or so species recorded in Africa. Hence the remaining three volumes would have had to cover 1,600 species, or twice as many per book as Vol.1. Even granted that this volume contains many of the better-known birds, such as the ducks and birds of prey, this would have been difficult. Fortunately the publishers have recently decided that there will be at least one further volume; this will ease things considerably.

The Birds of Africa, an immense project, was the brain-child of Leslie Brown who sadly died before this volume was completed. One very much hopes that the other authors will maintain his drive since the completed work will be a valuable contribution to ornithology. □

Christopher Perrins is Director of the Edward Grey Institute of Field Ornithology, Department of Zoology, University of Oxford.

A world of far away and long ago

Andrew Hill

The Historia Naturalis of Pliny the Elder. By Joyce Irene Whalley. Pp.46. ISBN 0-283-98905-X. (Sidgwick & Jackson: 1982.) £6.95.

PROPPED between two slaves poor Pliny died, while the pumice of Vesuvius fell about him. It was his constant search for facts that led to this early demise, a compulsion that had produced a number of books on a range of subjects, now all lost but for the encyclopaedic *Historia Naturalis*. This work justly purported to encompass ". . . the world of Nature, or, in other words, Life . . .", and assembled what Pliny calculated as 20,000 facts, culled from 2,000 other books and his own experience. Including places, peoples, animals, plants, geology, medicine, painting, it provides a rich view of the world in the first century AD. This is a significant view, for it was one that with all its faults and pseudodoxies dominated Europe through the Middle Ages and extended its influence to more recent times.

Joyce Whalley's fascinating work is rather less ambitious, being intended primarily as a picture book. In the 1460s Giuliano Amedei illustrated a manuscript of Pliny with elaborate historiated initials for each of the 37 books or chapters. These depicted scenes related to the subject matter, and occasionally are as fanciful as parts of the text. One, for instance, shows a varied assemblage of humanoids, including the rare umbrella-foot tribe who, disposing themselves upon their backs, avoid the sun's glare through the protection afforded by the shadows of their substantial feet.

Some of these beautiful illustrations, highly ornamented in white vine decoration, resemble secular aspects of the contemporary books of hours, being lively and realistic treatments of everyday fifteenth-century activities. Many also reflect Pliny's patchy accuracy: elephants have lions' feet, and even more readily observable insects have eight or more legs.

Amedei comments visually on Pliny; Joyce Whalley comments on them both. Short descriptive sections opposite each plate indicate their significance, giving a necessarily brief account of the appropriate chapter and selecting phrases, practices and beliefs that have some particularly modern association. This pleasant and enjoyable book provides a vivid and instructive glimpse of the world through earlier eyes. It is also an enticement to explore in more detail the complete work, forming a gentle introduction to the cosmos and nature of Pliny. □

Andrew Hill is a Research Fellow at Harvard University.

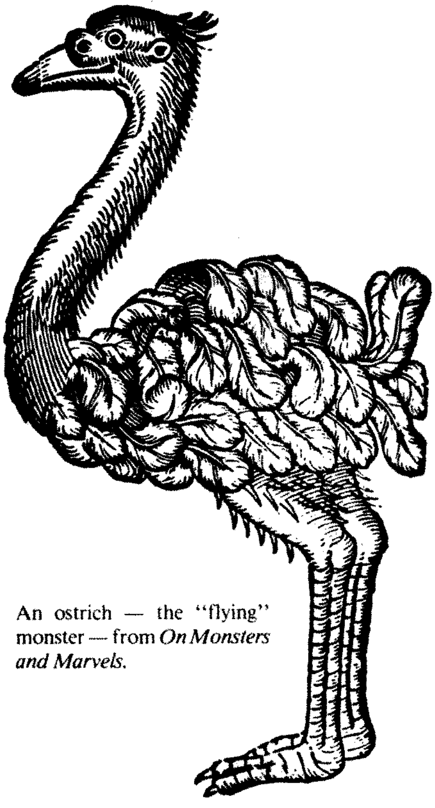
Creatures of fact, creatures of myth from the sixteenth century

Bernard Wood

Ambroise Paré: On Monsters and Marvels. Translated and introduced by Janis L. Pallister. Pp.224. ISBN 0-226-64562-2. (University of Chicago Press: 1982.) $20, £14.

IF ANY man has claim to be regarded as the Father of Modern Surgery, it is Ambroise Paré, the greatest of the Renaissance surgeons. Born of a humble family in 1510, Paré became apprenticed as a barber-surgeon, eventually moving to Paris in 1533. He gradually freed himself from his hair-cutting duties and was appointed *aide-chirurgien* at the Hôtel-Dieu, and by the age of 30 had qualified as a barber-surgeon.

Paré's training was essentially practical rather than scholastic; like many distinguished surgeons, he learned much of his craft on the battlefield. His first experience of war was the attack on Turin by Francis I in 1537. He quickly put his experience at the Hôtel-Dieu to good use, and concluded that the application of boiling oil frequently impeded rather than facilitated the healing of missile wounds. His campaigning days ended in 1559, but from then until his death in 1590 Paré managed to remain close to the crown and true to both the art and science of surgery. He advanced treatments as diverse as those for hernia and bone fracture, and devised enduring instruments such as forceps for clipping arteries.



An ostrich — the "flying" monster — from *On Monsters and Marvels.*

Of all his legacies, however, perhaps the richest is the breath, wisdom and humanity of his writings. He wrote not in Latin, but in Old French, and his willingness to discuss any matter on which he had practical experience did not always endear him to his peers. The 1575 edition of his collected works particularly outraged Estienne Gourmelen, the Dean of the Faculty of Physicians in Paris. Paré's reply to Gourmelen was trenchant and typical: "Dare you teach me Surgery, you who have never come out of your study? Surgery is learned by the eye and the hands. You mon petit maître, know nothing else but how to chatter in a Chair".

It is an indication of Paré's prodigious energy that *Des Montres et Prodiges,* first published in 1573, was the nineteenth in his series of surgical writings. Though the work had earlier between translated into Latin and thence into English, this edition is the first to be prepared by translating the original French into English. Janis Pallister's translation preserves the earthiness of the medical literature of the time — the directness and freshness of Paré's prose is in clear contrast to that used in modern-day texts where a leaden style is too often mistaken for scholarship. The dust-jacket claims that the edition includes a scientific commentary. It is true that a physician and a biologist provide notes and suggest identifications or diagnoses of what Paré describes, but these are modest and often merely expand on an earlier commentary by Paul Delaunay.

On Monsters and Marvels, a delightfully whimsical book, is divided into four parts. The first (and much the largest) section is devoted to human and animal monstrosities. Some of these animal "monsters" are descriptions and reports of creatures which are recognizable; others are clearly creatures of the imagination. The first chapters, devoted to human congenital and birth defects, make fascinating reading when one considers the period when the book was written. They include a description of what appears to be an example of the testicular feminization syndrome, and picturesque accounts of malingerers and patients suffering from calculi.

Flying, terrestrial and celestial monsters occupy the last three chapters. The "flying" monster turns out to be the ostrich and the terrestrial one the elephant, and the structure and habits of both animals are described and discussed: the observations on the ostrich were based on a skeleton of one of three ostriches kept by King Charles. The suggested identifications and scientific notes are useful but not infallible. For example a stone "under the tongue" of Captain Augustin, Engineer to the King, was almost certainly in the duct of the submandibular gland and not in the duct of the parotid as is claimed in the commentary.

It may seem to us that a book which dwells on monsters and marvels was an idiosyncratic choice for a surgeon. However in Paré's time such books were in vogue, and we must be grateful to Janis Pallister for making this curious and beguiling work more widely available. ☐

Bernard Wood is Professor of Anatomy at The Middlesex Hospital Medical School, University of London.

Fragments of the past

John Sutton

A Geological Miscellany. Compiled by G.Y. Craig and E. Jean Jones. Pp.216. ISBN 0-946193-00-2. (Orbital Press, PO Box 141, Oxford: 1982.) £8.95.

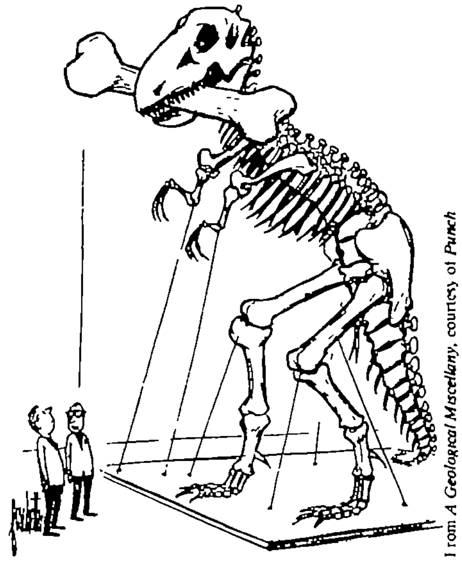
FOR many years it was the pleasant custom of the Geological Society of London to present new Fellows with Woodward's history of the Society's first hundred years. I know of no other book which conveys so effectively the liveliness and fun of those who set geology on its way early last century. Babbage in 1830 wrote of the discussions at the Society that "to say . . . they are very entertaining is the least part of the praise which is due to them".

To my mind Professor Craig and Mrs Jones might have done more to bring out the ability of so many pioneers to do serious work in a light-hearted and enjoyable way, and to convey the excitement of a youthful science. True, they have collected some nuggets, but the grade of the ore they have worked varies greatly and some of the longer of the 130 or so passages in this miscellany might have been consigned to the tip heap in favour of better material.

The two editors nevertheless should be congratulated on their happy thought of bringing together "a pot pourri of adventure, anecdote, epigram, autobiography, discovery, hypothesis and bureaucratic absurdity". There is plenty of material available, for geologists are gregarious people who pass on good tales from one generation to the next: it is still possible to encounter Fellows of the Geological Society who remember Archibald Geikie, who had been out in the field with a geologist who in turn had been on a geological excursion with James Hutton, born in 1726.

Craig and Jones have cast widely over the English-speaking world from New Zealand to New England and even beyond to Pliny on the destruction of Pompeii.

Among the treasures they have come up with are verses by J. Maynard Smith on the "Danger of Being too Clever" (part of a collection presented to J.B.S. Haldane) and a poem by Edward Forbes addressed to a lady whom he must subsequently have left for another. We encounter Edgeworth David suspended over an Antarctic crevasse yet too polite to disturb his only companion, Mawson, who was changing photographic plates unaware of Edgeworth David's predicament. We hear of Ami Boué declining an offer of six Bulgarian maidens when in the field early last century, and of Mary Anning's exchanges with the King of Saxony. Mention of royalty brings to mind one of the best geological passages, an account by Queen Victoria of the parallel roads of Glen Roy.



From A Geological Miscellany, courtesy of Punch

"It seemed logical. We had one bone left over."

These are good historical vignettes; and there is genuine scientific interest in a letter from Sedgwick to Darwin expressing his reservations on the *Origin of Species*, and in an equally sceptical reaction from a Geological Society of America audience to Griggs's paper on convection currents in the mantle. More as good as these would have been preferable to the accounts of swindlers in nineteenth-century USA, of an alcoholic drive through New Mexico and of diamond hoaxes in England which together take up a tenth of the book. And it was insensitive to guy Edward Greenly's tribute to his wife.

All in all, my verdict is — a good idea, could have been carried out more effectively. Let Johnson (quoted p.161) have the last word. Boswell: "Is not the Giants' Causeway worth seeing?". Johnson: "Worth seeing? Yes, but not worth going to see". That sums up the book. ☐

John Sutton is Pro Rector and Professor of Geology at Imperial College, University of London.

More of dinosaur extinction, and more

Malcolm C. McKenna

Hunting the Past: Fossils, Rocks, Tracks and Trails — The Search for the Origin of Life. By L.B. Halstead. Pp.208. ISBN 0-241-10899-3. (Hamish Hamilton/Doubleday: 1982.) £10.95, $19.95.

Beverly Halstead is probably most familiar to readers of *Nature* as the instigator of various criticisms of scientific work at the British Museum (Natural History) and of its epistemologically exciting exhibition policies. Polemics apart, he has also published some very important research on Palaeozoic fishes and on vertebrate hard tissues.

In *Hunting the Past* he shows us some of the wonders of geology, palaeontology and physical anthropology, explained simply and with apt and captivating illustrations, most of which are in colour. The book is aimed at beginners but focuses more on geology and on the romance and history of fossil hunting than on how scientific studies are conducted once the field-work stage is finished. *Hunting the Past* is in the *Time–Life* tradition, a book for the intelligent layman but not for the edification of advanced amateurs or professionals.

Much emphasis is given to the ever-popular dinosaurs. The sections on speed and body temperature are welcome improvements over most of the older popular treatments, in view of the obvious complexity of the subject and Halstead's need to simplify in the space available. At several places in the book, however, the reptile-like mammals (therapsids) are dubbed paramammals, an unnecessary redundancy. One wonders why the dinosaurs were not dubbed parabirds!

On the subject of dinosaur extinction, Halstead discounts the currently fashionable asteroid impact hypothesis, noting correctly that many dinosaurs were already extinct by the time of the Cretaceous–Tertiary impact event and its associated iridium anomaly and disruption of the calcareous marine biosphere. No one doubts that crashing asteroids would have had no effect on already extinct organisms, but the argument that the extinction of the very last of the dinosaurs is close enough to the K–T boundary to coincide with it is now being championed with renewed statistical evidence by Louis Alvarez.

Further, anti-asteroid arguments that dinosaur extinction was diachronous from north to south rest heavily on palaeomagnetic polarity stratigraphy that is itself suspect. Anomalies may be missing from the stratigraphic record of the San Juan Basin of New Mexico because of a mistaken lack of notice given to a late Cretaceous to early Palaeocene unconformity beneath the restricted Ojo Alamo Member of the Nacimiento Formation. Moreover, Archibald recently reported a lignite ("Will the Real Z Coal Stand Up") beneath the famous Bug Creek Anthills site in Montana. There are even rumblings that dinosaurs have been found above what is identified as the Z coal elsewhere. The fat is likely to remain in the fire for some time while all this confusion is sorted out. Meanwhile, Halstead is unwise to play down the asteroid impact hypothesis as a cause of extinction of the last of the parabirds.

In the anthropological part of the book the Piltdown Hoax caught my eye. Even the Loch Ness Monster and Glen Rose Cretaceous "human" footprints from Texas get space. And of course there are some amusing errors and misprints, such as Powell supposedly discovering the Grand Canyon, the multituberculates being categorized as rodents or the apt description of Glen Rose footprint modifiers as "Cretationists".

At the end of the book there is a section on classification in which, true to form, Halstead misinterprets cladistics. He seems to have read Hennig's 1966 book, but the subject has progressed a bit since then. In all, Halstead's attempt to purvey palaeontology and geology to a non-professional audience is a creditable effort — but it falls far short of excellence. ☐

Malcolm C. McKenna is in the Department of Vertebrate Paleontology at the American Museum of Natural History, New York.

Going to ruins in central America

Warwick Bray

The Complete Visitor's Guide to Mesoamerican Ruins. By Joyce Kelly. Pp.527. ISBN 0-8061-1566-1. (University of Oklahoma Press: 1982.) $35.

There have been, as the saying goes, plenty of guide books to Mesoamerican ruins, but never one like this. The author covers 119 archaeological sites (graded from one to four stars, in terms of viewability) and also 41 museums in Mexico, Guatemala, Belize, Honduras and El Salvador. A "Complete Guide" it is not, and every reviewer will lament different omissions (I particularly regret the Cacaxtla murals and the new excavations at the Aztec Templo Mayor in Mexico City), but all the principal sites are included, as well as an enterprising selection of second quality sites in the Maya zone and Pacific Guatemala.

Information on the cultural background to the architecture is rather sketchy, and technical terms are occasionally used without explanation. On the credit side, the book is lavishly illustrated with photographs, but would benefit from more detailed site maps, and has only a short and selective bibliography at the end. For each individual site the author includes a section (sometimes quite lengthy) on the history of discovery and research, giving the names of the excavators, with their institutional affiliations and the dates of their investigations, but not the full bibliographical references that would carry the serious amateur forward to the next level of reading. This merely causes frustration, and was a serious error of judgement on the part of the author or publisher.

The introductory chapter contains much good general advice, on everything from camera equipment to car insurance, but is strongly slanted towards the North American reader. British tourists should check their own visa requirements and, with the collapse of the Mexican peso, should watch out for changes in currency regulations. Probably, too, El Salvador is a place to keep away from at the moment.

The emphasis throughout this book is on how to get to the sites and what to see there. The "ruin buff" (a favourite phrase of the author) to whom this advice is directed comes over as rather timid and of a certain age, driving his — or more probably, her — own car, worried about snakes, insects, sunburn and where to find the nearest cold drink, and in a perpetual dither about whether to wear boots or tennis shoes. Carrying a book of this size, too big and heavy to fit the pocket, walking is going to be hard work. Eye make-up tends to run, but if it rains you can always "carry a wig to wear when you get back to the cities or until you can get to a beauty shop". The main purpose of the visit is not to study aboriginal culture, but to get the best possible photos (or, at least, the same ones that everybody else takes) in the shortest possible time.

It's easy to poke fun, but this is a perfectly legitimate market to aim at. As a visit to any major ruin will demonstrate, there are a lot of people just like this (so many that they tend to get in the way of each others' photos), and they very often need precisely the kind of help that Joyce Kelly's book provides. So, too, do the more snobbish, those of us who through professional arrogance or sheer carelessness have got lost, gone hungry or arrived at a site to find the main buildings in deepest shadow. Thanks to Mrs Kelly, we should not make those mistakes again.

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Review supplements for 1983

Dates of the five special review issues to be published by *Nature* in 1983 are: Textbooks, March 10th; Spring Books, April 28th; Journals, October 6th; Autumn Books, November 10th; and Christmas Books, December 1st.

Wiggles put straight

Peter J. Smith

Inside the Earth: Evidence from Earthquakes. By Bruce A. Bolt. Pp.191. Hbk ISBN 0-7167-1359-4; pbk ISBN 0-7167-1360-8. (W.H. Freeman: 1982.) Hbk $22.50, £17.80; pbk $11.95,£9.95.

PROFESSOR Bolt has dedicated his latest book not only to the pioneers of seismology in general but to Richard Dixon Oldham in particular, and with good reason. Though little remembered these days, it was Oldham who first showed, in 1906, that earthquake waves could be used to demonstrate the existence in the Earth of major physical discontinuities and thus of a large distinct central core. Indeed, the reading of Oldham's paper on the subject to the Geological Society was a far more significant event for the Earth sciences than the now better-known San Francisco earthquake that was to follow just a few weeks later, for although the disaster in California undoubtedly gave both a scientific and a political boost to the study of seismology, it was Oldham who enabled such study at last to be "removed from the realm of speculation into that of knowledge".

The particular dedication is also appropriate in a quite different sense, even if Professor Bolt would not say so himself. Oldham's 1906 paper was a model of exposition; and in his own way and with far less tractable material Professor Bolt hardly achieves less. There are, for example, few things in seismology more difficult to explain to the non-specialist or undergraduate than terrestrial spectroscopy and few things more mind-boggling than trying to envisage the overtones of the spheroidal and toroidal eigenvibrations of the Earth. After reading Professor Bolt's 18-page chapter on these free oscillations, many may well feel that some of the minor mathematical and notational details elude them; but they will hardly come away with less than a very firm grasp of the physical principles involved. To be able to impart a strong sense of what is actually going on, as opposed to merely presenting some obscure mathematical description, is no mean achievement.

On the principle that anyone who can successfully explain the nature of the Earth's free oscillations can explain anything, it comes as no surprise to find that Professor Bolt maintains the same high standard of exposition in dealing with the rather less exotic, but no less interesting, aspects of seismology — the nature and measurement of earthquake waves, the significance of wave paths and travel times, the interpretation of seismograms in terms of broad Earth (and lunar and Martian) structure, the elucidation of finer (transition zone) structure, the estimation of density from seismic properties and so on.

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Peter J. Smith is Reader in the Department of Earth Sciences at the Open University, Milton Keynes, and editor of Open Earth.

Carbon dioxide and climate (and journalism)

John S. Perry

Future Weather and the Greenhouse Effect. By John Gribbin. Pp.302. ISBN 0-440-02498-6. (Delacorte Press/Eleanor Friede: 1982.) $15.95.

JOHN GRIBBIN essays a formidable task: to explain the genesis, mechanisms, history and probable future course of Earth's climate in some 300 pages of non-technical prose. His presentation is admirable in its design and broad thrust, but he has left some nasty booby traps for the unwary reader.

Over the lifetime of our planet, atmosphere, climate and life have evolved together. Climate has varied throughout the long history of the globe, and the short history of man. Rapid changes in climate could have drastic consequences and are a legitimate cause for concern in a world already uncomfortably dependent on crops from a few climatically favoured lands. The most important human influence on climate is likely to be an increase in atmospheric carbon dioxide from the combustion of fossil fuels; models of the Earth's atmospheric and oceanic systems project significant changes in the workings of the great heat engine that powers our winds and governs our climate. Future weather will be different, and mostly warmer.

However carbon dioxide "feeds" plants, farmers are clever and people will probably continue to starve or grow fat primarily because of society's aberrations, not those of the weather. Changing climate — whatever its origin — simply complicates our task of providing energy, food and a decent life for everyone on Earth.

Communication of this thread of understanding to the public would be a noble achievement. Indeed, Gribbin's attempt has certain points to recommend it. He wields a breezy, conversational style and has a journalist's eye for arresting details of personality and event. Moreover, his explanations of certain scientific concepts and research results are outstandingly clear and accurate. For example, his discussion of the greenhouse effect is a model of its kind, and the core of his outline of the general circulation is probably as good as can be achieved without recourse to mathematics.

Unfortunately, other flaws sadly vitiate the book's value and betray its promise. The introductory chapters are crammed with a motley assortment of climatic titillations — droughts and blizzards and ice ages — each hurriedly and simplistically linked to one or another meteorological abstraction. Jet streams, the circumpolar vortex and the Intertropical Convergence really make sense only in the context of the general circulation, an insight postponed until Chapter 3. By that point, the lay reader may find himself mixed up and turned off.

The major failing seems to stem from a mismatch between the nature of science and the demands of journalism. There is a popular misconception that scientists are people who look for answers. That's nonsense, of course: although they generally share a working consensus, scientists are at root people who like to ask questions and pose hypotheses. Bearers of all sorts of ideas are eagerly welcomed to science's great and never-ending party, and the climate corner of the affair — one pictures it as lying between the hors d'oeuvres and the bar — is particularly crowded.

Thus, climate vies with politics, economics and religion in the number and variety of theories per unit of fact. All attempt to explain some aspect of the behaviour of a complex and poorly observed heat engine employing turbulent working fluids, subject to changing forcing, and tampered with to an unknown extent. Variability on all time-scales is a natural and inherent characteristic of climate, so it is no wonder that plausible connections can be adduced between almost any hypothesized cause and virtually any climatic consequence. Few survive independent data and scrutiny, yet Gribbin appears ready to embrace almost any theory — one longs to sell him a used car. The Sun–weather relationships, for instance, to which many pages are devoted, have proven particularly fragile, and it is misleading to treat these empirical speculations as established facts. Physics should rule over statistics, and a treatise on climate should seek to present a sound consensus and be built upon critical scientific judgement.

Instead, the book seems to have been constructed with the help of a vacuum cleaner and a glue pot. One imagines the author pointing his nozzle into all the dusty corners of the village library and cheerfully pasting up with unbounded enthusiasm whatever comes down the pipe — trash and treasure alike. One scientist's solar-based forecasts have "invariably proved right", a judgement shared by few and contradicted a page later. The atomic bomb and man-made aerosols are brandished as precursors of an ice age. For some reason, one dubious claim of geomagnetic-climatic connections merits an entire chapter, while another naive misinterpretation of irrelevant observations "commands respect" in assessing CO_2-induced climate changes. It is astonishing that Gribbin, a physicist by trade, swallows an outrageous claim that even an atmosphere totally opaque to thermal radiation could warm the surface by no more than 4°C. Physics aside, hasn't he ever heard of the heat wave on Venus?

Future Weather and the Greenhouse Effect has many ingredients of a badly needed commodity — a well-balanced and comprehensive book on climate for the layman. The man in the street, however, would be best advised to look elsewhere for a balanced understanding of knowledge and speculation is this important and exciting area of science. □

John S. Perry is Executive Secretary to the Board on Atmospheric Sciences and Climate, National Academy of Sciences, Washington DC.

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A universe without mathematics

Hermann Bondi

The Lighter Side of Gravity. By Jayant V. Narlikar. Pp.194. Hbk ISBN 0-7167-1343-8; pbk ISBN 0-7167-1344-6. (W.H. Freeman: 1982.) Hbk $17.95, £13.95; pbk $9.95, £6.95.

JAYANT Narlikar's *The Lighter Side of Gravity* is a splendid effort to bring together many of the most exciting facets of physics and astronomy in a logical argumentation devoid of mathematics. Much so-called popular writing on physical science is content with a description of where today's science has got to; moreover, such a description is often marred (particularly in the media) either by delighting in stressing how "crazy and incredible" it all is, or by regarding the development of science as a sort of intellectual stock exchange where individual scientist's stock rises and falls according to how "right" or "wrong" their views have turned out to be (as though a scientist's standing depended on this!).

Narlikar is wholly free from these faults. He respects his reader and takes him gently, almost imperceptibly, through many of the important arguments leading to the advanced concepts of black holes, of the source of stellar energy, of white holes and of the structure of the Universe. He is outstandingly successful in this, though I feel that some of his derivations are a trace old-fashioned in their nature and in the analogies used. His clear writing, stressing how intelligible it all is, is a delight and indeed a breath of fresh air in contrast to so much of the sensation-hungry writing in this field.

There can be no doubt that this book meets an important need and meets it very well indeed. Yet one must admit that it is a curious aberration of our educational system that the need for such a book without mathematics is so great. Mathematics is used in physics and in astronomy to make arguments easier, to make the work lighter. Yet we create such a phobia of equations and formulae in the bulk of the population that there is a continuing demand for books that try to make the going easier by explicitly *excluding* mathematics. This is no fault of the author or of this splendid book, but rather a fault of us all for engendering in so many thinking people a violent reflex action to anything mathematical. ☐

Sir Hermann Bondi is Chairman of the Natural Environment Research Council. His books include The Universe at Large *(Heinemann, 1961) and* Assumptions and Myth in Physical Theory *(Cambridge University Press, 1967).*

Sky-watchers A to Z

Stephen P. Maran

Observatories of the World. By Siegfried Marx and Werner Pfau. Pp.200. ISBN 0-7137-1191-4. (Blandford/Van Nostrand Reinhold: 1982.) £8.95, $16.95.

IN *Observatories of the World* two astronomers at the University of Jena tell tales of 40 of their favourite observatories from Abustumani to Zelenchukskaya. The observatories have been selected to represent institutions of different types, including those that are research-orientated and education-orientated, those with optical, radio and infrared intruments or specializing in elaborate computational tasks, and those located in cities, on mountaintops or (in one case) aboard an aircraft. The English-language translation is by Simon Mitton, who also revised the text.

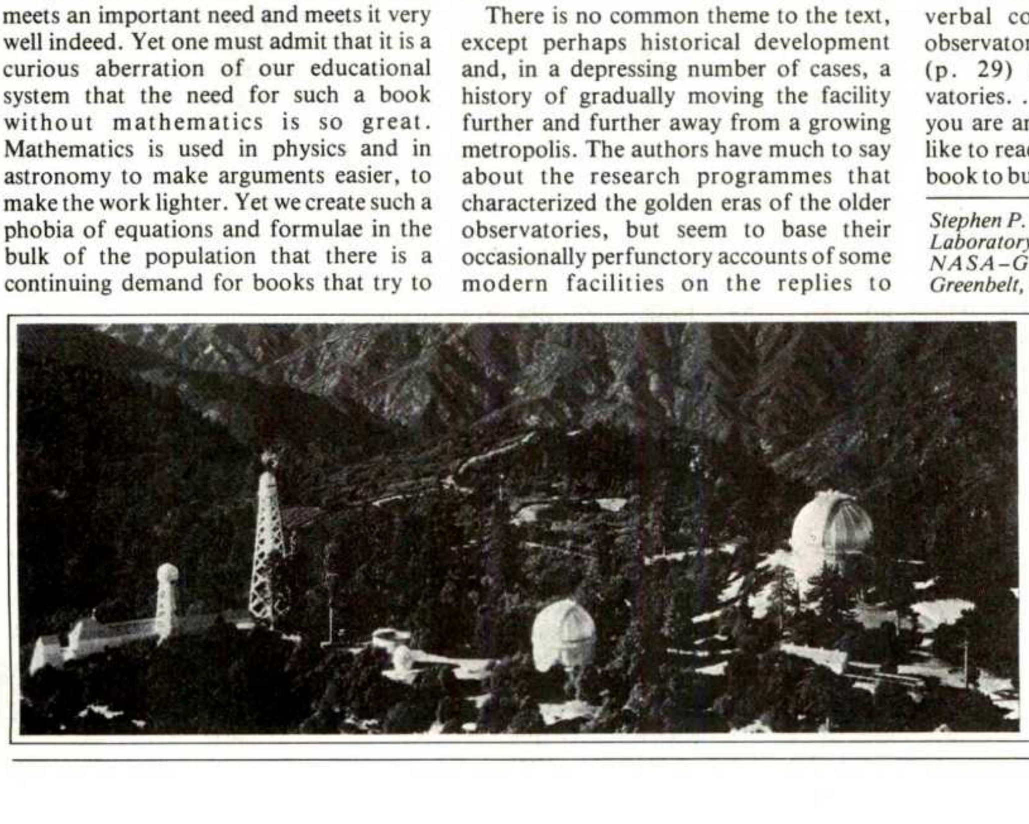
There is no common theme to the text, except perhaps historical development and, in a depressing number of cases, a history of gradually moving the facility further and further away from a growing metropolis. The authors have much to say about the research programmes that characterized the golden eras of the older observatories, but seem to base their occasionally perfunctory accounts of some modern facilities on the replies to questionnaires that were distributed to the observatory authorities. This is not a professional reference work, then, nor a directory; there are no tabulations of telescopes and instruments, nor of observatory coordinates and altitudes.

Not all of the facilities described would have made my "Top 40"; the omission of Yerkes Observatory (mentioned in passing, and incorrectly, as being located "in the East of the United States") is particularly unfortunate for it was at Yerkes that much of modern astrophysics was born. Cerro Tololo Inter-American Observatory, that great and much-used telescope farm in Chile, is also not included, although it is briefly described in the section on its sister facility at Kitt Peak.

Observatories of the World is written for those who have some familiarity with the terminology of astronomy, although a number of elementary concepts are explained. It is extensively illustrated with attractive half-tones and rather primitive sketches (plates 11 and 12 appear to be interchanged, incidentally, but most readers should be able to tell a coronagraph from another kind of telescope). Only here, among books that come conveniently to mind, can one readily determine that the ancillary building of Konkoly Observatory near Budapest "has a grass-covered roof and cannot therefore be a source of troublesome air turbulence. . ." or that the little town of Coonabarabran provides scientists with "all the comforts of civilization". Amidst the interesting trivia, however, there is a brief but frank discussion of optical problems with the original 6-metre primary of the huge reflector at Zelenchukskaya, and many other informative titbits.

The overall result is a pleasant and informative book, despite some awkward verbal constructions such as "Every observatory not recently established. . ." (p. 29) where "The older observatories. . ." might have done as well. If you are an armchair astronomer and you like to read about observatories, this is the book to buy. ☐

Stephen P. Maran is Senior Staff Scientist in the Laboratory for Astronomy and Solar Physics at NASA–Goddard Space Flight Center, Greenbelt, Maryland.



Mount Wilson Observatory in southern California. To the left are three solar telescopes, centre and right are the domes of the reflectors. The picture is taken from Richard Learner's *Astronomy through the Telescope,* recently published by Evans/Van Nostrand Reinhold. Prices are £12.50, $29.95. The excellent illustrations and accessible but comprehensive text make the book a stimulating guide to the history of astronomy and its instruments for both professional astronomer and layman.

The cosmos and its contents

David W. Hughes

NEW BOOKS on astronomy continue to flood on to the market, the fact that we are not swamped by them being due to their short life; few last more than two years before going out of print or finding their way onto the remainder shelves. The astronomical second edition is indeed a rarity. Nineteen eighty-two, however, does have advantage over other years — it is the twenty-fifth anniversary of the start of the space race. My first book deals with just that.

Sputnik to Space Shuttle by Iain Nicolson (Sidgwick and Jackson, £7.95) chronicles the childhood of the space age. We start with the characteristic "bleep bleep" of the Soviet Union's Sputnik 1 which astonished the world on 4 October 1957 and end with the graceful landing of Columbia which completed the fourth test of the Space Shuttle in June of this year. Between these dates there is all the excitement of the race into space, the Soviet Union and the United States continually jockeying for the lead. First dog in space, man in space, woman in space and space walk went to the USSR; first voice from space, navigation satellite, meteorological satellite and communications satellite went to the USA; first photographs of the far side of the Moon, first lunar soft-landing, first Venus soft-landing to the USSR; first men on the Moon, first two-planet mission, first Mars lander to the USA. One could continue but why not just read Nicolson's book. He is a lively writer with a clear, easily readable style and his book is an excellent review of our 25 years "out there".

For a more scientific bias we have **Planets of Rock and Ice** by Clark R. Chapman (Charles Scribner's Sons, $13.95), a revised and expanded edition of his 1977 book *The Inner Planets*. The author folds together our knowledge of terrestrial planets with the new-found information gleaned from the space probe observations of the satellites and rings of Saturn and Jupiter. This book is raised above the mundane plane of the usual account of the planets by the many anecdotal insights into the workings of the mind of a planetologist that grace its pages. Chapman loves his job, loves the planets and it shows.

In the book he inveighs against the shut down of Solar System explorations that has been inflicted on the American scientific community. There is, however, a very minor advantage to this in that scientists will no longer be distracted by the "gee whiz" findings of new planetary encounters or the need to plan for forthcoming missions. So the storerooms of accumulated information, the thousands of pictures and unprocessed magnetic reels of data, now have a chance of being sifted and scrutinized. Be this as it may, there are still a multitude of places to go to and experiments that cry out to be performed.

The evolution of Earth's surface and atmosphere and the way it contrasts with its rocky neighbours is obviously of great interest and concern to Chapman. His ability to express this vividly makes the book a joy to read. But how different is the state of planetology today in comparison to the time when Apollo 11 landed. The London bookmakers William Hill were then offering odds of 100:1 against a manned Mars landing before 20 July 1976 and "evens" on a landing before 20 July 1979!

The Planet Venus by Garry E. Hunt and Patrick Moore (Faber & Faber/Faber Inc.; £10, $22) divides neatly into two parts. Venus before the space age is mainly written by Patrick Moore and concentrates on the way Earth-based telescopic and spectrographic observations moulded our pre-1962 view of the planet. Transits, the Black Drop problem and the ashen light are carefully reviewed, as are more marginal and esoteric phenomena such as the debate that took place in the 1950s as to whether the surface was covered with oil, water or dust, the aphroditographic work of Percival Lowell that "revealed" a series of dark patches and strips on the planet and a rotation period synchronous with the orbital period, the discovery of a phantom satellite and its subsequent demise, and the early attempts at measuring the surface temperature.

Venus made its entry into our space age in December 1962 when Mariner 2 flew by, and the voyages of discovery have continued with a whole series of Venera, Mariner and Pioneer spacecraft. Garry Hunt reviews the latest ideas about Cytherean greenhouse effects, atmospheric sulphur cycles, wind profiles, circulation patterns, and day and night variations. Pictures are shown of the surface of Venus near the Venera 9, 10 and 13 spacecraft, and the results of the radar observations of the landscape using data from the Pioneer orbiter and the Arecibo and Goldstone radar facilities are reviewed.

Venus is often regarded as the Earth's twin but an identical twin it certainly is not. This book is a very good account of our knowledge of our nearest planetary neighbour; I was especially impressed by the extensive reference section which will encourage readers to delve further.

Now for something completely different. What started in the United Kingdom in 1767 and the United States in 1855? It was the forerunners of the **Astronomical Almanac** and the 1983 version (US Government Printing Office/HMSO; $16, £9.25) would make an excellent gift for the astronomer who has nearly everything. Where else would you easily find the universal times of the greatest northern elongation of the satellite of Pluto close by lists of the 194 brightest galaxies (together with diameters, photometric data and velocities), the 1,482 stars brighter than 4.5^m, the latitude and longitude of the world's optical observatories, the circumstances of all the 1983 eclipses, together with a host of all the usual things we refer to the almanac for? Many happy hours can be spent dipping into this book.

To move closer to the stars, **The Astronomy Handbook** by James Muirden (Kingfisher/Archon; £3.95, $8.95) is a colourful, profusely illustrated guide to the constellations, Sun and Solar System. It is aimed at the teenage astronomer. Muirden concentrates on the practical side of the subject and I really like his plan for a do-it-yourself planetarium — no young astronomer's bedroom should be without one. The book is full of encouragement, more than enough to make any youngster grab a pair of binoculars, brave the cold of a starry night and take to examining the heavens.

Planets first, stars next and finally the Universe and all the theories and phenomena of the cosmos. **Deep Space** by Colin Ronan (Macmillan, London/Macmillan, New York; £10.95, $25.95) and **The Restless Universe** by Nigel Henbest and Heather Couper (George Philip, £8.95) both throw their net wide and try and sweep in the whole zoo of astronomical objects from pulsars, quasars and black holes down to stars and planets. Both delve into the mysteries of the big bang, the search for alien life, how it will end (the big crunch?), and the birth and death of stars. It is the differences between the two books that are really startling. Ronan's book is a visual treat and is profusely illustrated not only with photographs of celestial objects but also with a host of well-conceived figures: for example, one diagram shows the relative positions of the brightest members of the Local Group of galaxies and with the accompanying table can form the basis of a model; another does the same for the Sun's nearest 24 stellar neighbours. It is very hard to resist making these models. I gave in straightaway and had great fun. Time and again the illustrations really punch home the message of the text. The figures are exciting, the text interesting and I have no hesitation in recommending Ronan's book.

Henbest and Couper confine themselves to 24 figures and 24 colour photographs and I feel that the authors have set themselves the age-old problem of describing a spiral staircase with their hands in their pockets. This comment apart it must be admitted that the text rattles along at a heady pace — nobody will fall asleep reading this book. ☐

David W. Hughes is a Lecturer in Astronomy and Physics at the University of Sheffield.

nature

16 December 1982

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Human guinea pigs have a choice

The British Medical Research Council is to carry out a five-year, controlled trial of vitamin supplements in the prevention of neural tube defects. But it should still adopt more open discussion of the trial.

Should people ever be used as guinea pigs in the investigation of a new drug or some new medical treatment? That is one of the questions on which opposition has been focused in the past few months to the proposed British trials of the efficacy of vitamin supplements, including folic acid, as a means of preventing neural tube defects (including spina bifida) in human births. The simple answer to what is an over-simple question is yes. People should be used as if they were experimental animals in proving new medical treatments. Moreover, people are already being used widely for just that purpose. But the analogy with guinea pigs is false. Guinea pigs are not able to choose whether to be part of some controlled trial in the laboratory. People invited to help test some novel treatment are, on the other hand, first given an explanation of what is intended and then an opportunity to decide whether or not they will participate. This is why it is right, if nevertheless courageous, that the Medical Research Council should have decided to go ahead with its long-planned trial of vitamin preparations as a means of preventing the recurrence of spina bifida births.

The planned trials are nevertheless unusual for several reasons. The incentive is the appearance in the past few years of evidence to suggest that supplements of vitamins, including folic acid during the first third of pregnancy, will help to prevent the recurrence of neural tube defects. The evidence (see *Nature* 2 December, p.396) is impressive but not conclusive. The planned trial is therefore based on the choice of four groups of women, each of whom has already given birth to a defective child, to which would be administered four different treatments — folic acid alone, folic acid plus more familiar vitamins, vitamins without folic acid and, for the remaining group, a placebo devoid of vitamins. The objective, of course, is not merely to discover whether one of these treatments will reduce the recurrence of neural tube defects but also to find some way of avoiding these defects among all women embarking on pregnancy. The controversy about the trial centres on the presence of the placebo group. Why, the critics ask, deprive one quarter of the women in the trial of any treatment when there is all this evidence that something in the original vitamin cocktail has some effect?

The assumption underlying this question is unfortunately just that. The control groups in the previous studies have not been control groups at all, but merely groups of women who have already had one defective child and who might have been offered vitamin supplements if they had not become ineligible, usually by already being pregnant (and only occasionally by refusal). That there may be some kind of self-selection in the assignment of women to the two groups is clear, although it is unlikely to have been significant. But certainly the women not given vitamin supplements in previous surveys, as in that now being carried out in the Republic of Ireland, are denied the benefits of the well-known Hawthorne effect, the improvement of virtually any social condition that comes about when something new is offered by experimentalists. Consequently, a trial without a placebo group would literally be incapable of yielding results bearing on the benefits that would obtain from the widespread administration of vitamin supplements to all women at risk of pregnancy, not merely those unfortunate enough to have produced an afflicted child. The point is especially important because there appears to be very little direct evidence of the untoward effects that there may be of chronic administration of folic acid, and because it may in the end be necessary to administer whatever vitamin supplement is necessary by adding it to some general component of the diet, bread perhaps. Will those who now complain that a placebo group would be unethical in the forthcoming trial then take to the hustings to protest that it is unethical to add an untested material to everybody's diet?

So the case for going ahead with the Medical Research Council's trial is strong. The obvious alternative approach, the detailed investigation of the concentrations of various vitamins in different kinds of body cells at different times of life, but especially in pregnancy, would no doubt be valuable (and should not even now be neglected). The case for an independent investigation of the effects of the chronic administration of folic acid in laboratory animals is strong, but as a necessary complement to the trials now planned. But none of this could possibly yield, in the near future, guidance on what should wisely be done to reduce the incidence of neural tube defects in the population as a whole. Only the trial can do that.

But will the Medical Research Council be able to recruit the physicians and their patients whose cooperation will be necessary after all the controversy there has been? The council's statement last week says that it is anxious on this point. The burden of advising women whether to take part in the trials will after all fall on family physicians, not on the research council's officials. One particular difficulty will be that women who are unable to accept that a fetus diagnosed as carrying a neural tube defect could be aborted will be reluctant to take part. For others, the crucial consideration will be whether the proposed trials will be accompanied by thoroughly reliable means of diagnosing neural tube defects early in pregnancy. The Medical Research Council should not be afraid that the provision of such arrangements will expose it to the scurrilous charge that it is prepared to offer abortions to women taking part in the trial. In these circumstances, as in the ordinary ups and downs of pregnancy, such questions are questions between women and their physicians.

None of this implies that the Medical Research Council will have an easy passage in the five years ahead. While the council has been attacked unfairly on many occasions in the past few months, it should itself acknowledge that it has helped to exaggerate its own difficulties by its attempts to control open discussion of its plans. The pantomimic suppression of two letters from *Nature* correspondents a few weeks ago (see 25 November, p.310) was thoroughly unreasonable; it was probably, in the event, counterproductive. In the coming months, the council should resolve to be more open about its plans. For example, the protocols that have no doubt been drawn up to help decide when the trial should be abandoned, perhaps because significant evidence has accumulated in one direction or the other, should be openly published. Tactically, and so as not to compromise its other good works, the council should remind anyone who will listen that it is not the prime mover behind the spina bifida trials but rather the agent of the Department of Health, which originally set the ball rolling. But, for the long term, the council should also insist not merely on the need for this planned trial but also on the fact that advanced communities such as the British cannot look for all the benefits of modern medicine if they shy away from the need for trials in which some people are put at risk.

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Ocean bed law disaccord

Several countries have not signed the Law of the Sea. A compromise must eventually be found.

The conference at Montego Bay in Jamaica to celebrate last week the signing of the Law of the Sea was by all accounts more like a wedding without the groom than a funeral without the corpse. The air of mutual congratulation was real enough. More than a hundred states turned up to sign the treaty; a third as many, including the United States and most of the industrialized states of Western Europe, stayed away. The issue that divides the two groups is now familiar: the arrangements in the treaty as negotiated for the exploitation of minerals on the sea-bed.

The disagreement is partly geographical. Within the newly defined "economic zone" of 200 miles around each maritime state, governments will be free to take what minerals they can find. Beyond that it will be necessary for those with the techniques for extracting minerals from the sea-bed to limit what they take but also to "share" their "technology" with other states that may share ambitions to win minerals from the deep oceans. While the provisions now put forward in the Law of the Sea are a good deal more realistic than those originally canvassed, they still give offence. The essential difficulty is that of making a bridge between the commercial interests of those who may in due course wish to retrieve minerals from depths of several kilometres, and who will presumably have to spend substantial amounts of their own money to reach a position in which they can do something worthwhile, and the objectives of those who are more concerned that the deep sea-bed should become a kind of international heritage of mankind. But too many of the schemes which are compromises between commercial interests and international idealism will work less than efficiently.

Several questions arise, the chief of which is that of who can be blamed for the way in which the Law of the Sea has not been signed by the potentially most important signatories. The impasse that has arisen was apparently well concealed at Montego Bay last week. The temptation now is to blame the United States for what has happened, and indeed the behaviour of the US delegation at the negotiations in New York earlier this year was a shameful mixture of indecisiveness and arrogance. But in reality what the Reagan Administration was saying that it would be a dangerous precedent if they were bound by the terms of an international treaty to share technology on terms unspecified and by means not understood and that the provisions of the Law of the Sea on deep-sea mining are merely a continuation by other means of the longstanding argument between the developing countries and those of the industrialized West. It would have been better for all concerned if these points had been made more openly much earlier in the negotiations.

So what happens next? Technically the treaty will come into force only when sixty of its signatories have taken the formal step of ratification, which is expected to take two years. Then, after a further five years, there will be a review conference at which the working of the treaty will be re-examined, and when it will be possible for those who have ratified the treaty to put forward amendments. Those not (repeat not) present at Montego Bay last week are now saying that they will nevertheless turn up at the review conference, if only as observers, to repair some of the damage that has been done. The snag is that without formal status as members of the treaty, they will be able to put forward new proposals only with the consent of the developing countries to which such offence has now been given.

Meanwhile, the signatories of the treaty are saying that as it now exists it is binding even on those who have chosen not to sign. The case for that position is weak. The signatories at Montego Bay last week may be right in saying that they could take non-signatory violators to the International Court of Justice at The Hague, but there is no reason to think they would win their cases.

So does the future hold merely a kind of licensed piracy on the sea-bed and even on the high seas? Given the likely pace of development of sea-bed mining, mineralogical piracy is unlikely. The provisions on the rights of innocent passage in narrow straits are more immediately important. The danger, in the present stand-off between the signatories and the others, is that their disagreement may become open dispute at times of international tension. Would the rights of passage through the Red Sea of merchant ships from non-signatory states always be assured if there were some future conflict in the Persian Gulf? This is the kind of anxiety that should keep the non-signatory governments awake at night, and on the look-out for a more persuasive compromise than they have found so far.

Unorthodox assessments

A recently published book provides an intriguing glimpse of peer-review in action.

Like restaurant proprietors, journal editors have the right to refuse admission to anyone, the obligation to explain their attitude being merely moral. Dr Stefan Marinov has therefore performed a useful and courageous service by revealing in print* the manner in which journals (including this one) and funding organizations around the world respond to this obligation. For some years, Dr Marinov has submitted papers and proposals concerning "absolute space-time" to such bodies, with — by his own account — a rather small degree of success. Literal copies of this correspondence make up the bulk of his book and are absorbing reading.

It is an inevitable part of the duties of every editor to assess papers that lie near what may be called the fringe of science. Physics journals and "general" journals receive many attempts to "correct" the theory of relativity or to propose new laws underlying the structure and evolution of the Universe. Some papers are based on numeralogical mysticism or are in some other way so speculative and unpredictive as to justify immediate dismissal. Others contain mathematical errors. Some (as in Dr Marinov's case) contain detailed arguments, often including descriptions of experiments, which require more lengthy assessment.

Dr Marinov's book reveals some of his arguments and the reception they received. As a result, it is something of a handbook for editors faced with the need for an appropriate choice of words if the assessment process has revealed that rejection is the appropriate course of action. Does one follow, at one extreme, the rather curt approach of *Science* ("We decline to publish your paper on 'Measurement of the one-way velocity of light and the Earth's absolute velocity'. The manuscript is enclosed.") or, at the other, does one follow the editor of *Zeitschrift für Naturforschung* and (as Dr Marinov relates) exchange more than twenty letters and give the author an explanatory conversation over a pizza? Between these two lie the responses of some 35 other journals and the National Science Foundation (four referee reports followed by formal appeals up successive rungs of the hierarchy).

What can or should be learned from such a book? Those as tenacious and as radical in their scientific approach as Dr Marinov will learn what to expect in tone and quality from the variety of journals available to them. Those responsible for journals or funding bodies will gain a glimpse normally denied them of their colleagues in action. They may be drawn to conclude that to refuse publication without providing justification is to do less than justice to any author and plays into the hands of conspiracy theorists. They will note with disquiet that the more conscientious they are in this respect, the more conscientious a reply they will probably receive.

Publishers might conclude that there is a market for an unrefereed journal of unorthodox science. All readers should note how, in this case at least, the system seems to have ensured that orthodoxy prevails — deciding for themselves whether by malevolent conspiracy, inertia or good sense.

**The Thorny Way of Truth*, Est-Ovest International Publishers, Via Puggia 47/1, 16131 Genova, Italy.

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Science to rescue EEC politics?

Davignon tackles the agricultural policy problem

Brussels

Scientific research in Europe seems poised to play a most unaccustomed role: as a tool in a major political issue, one which threatens the European Economic Community: the ballooning cost of the Common Agricultural Policy (CAP).

The CAP, designed in part to keep continental farmers on the land, consumes nearly three-quarters of the Community's budget, leads to enormous food surpluses, and threatens — politically — to blow the Community apart. Related policies of farming support in the United States also produce surpluses. Around 100,000 tonnes of butter, for example, are stored in a Kansas mountain; similar amounts cram cold stores in Europe. The consequent dumping of food on world markets is precipitating something approaching a transatlantic food trade war. Meanwhile, much of the world starves for want of rice, not butter or powdered milk, wine or maize.

The scientific initiative, designed to help solve the problems of CAP comes from the Vice President at the European Commission, Viscount Etienne Davignon — for whom everyone appears to jump when he wags a finger — and from his research director-general Professor Paolo Fasella. Between them they appear to be building the conceptual and political basis, both inside and outside the Commission, of a key role for research in agricultural policy, and, for that matter, in public health, which is another major financial headache for European governments.

These initiatives, still at an early stage (they are yet to be formally discussed by the 13 European commissioners), come after Davignon's success with Esprit, the Brussels programme for the information technologies. Davignon, assisted by Fasella's directorate, has steered Esprit through the labyrinth of EEC and national advisory committees, and around potential political opposition, with impressive speed. (The programme should start next year, only a year after conception. By comparison, an earlier and equally ambitious programme for biotechnology took seven years to reach this stage and is only just getting under way.)

The agriculture research proposals are to be based above all on a detailed analysis of the Community's agricultural budget — on what costs most and why. Beyond that, they relate — for the moment — to five central objectives.

The first objective is to reduce production and distribution costs, so that farmers can make their profit and maintain their living standards on a smaller volume of produce; the second, to upgrade the product; the third, to seek alternative uses of land and people; the fourth, to take account of the specific food needs of developing countries — or, more prosaically, the existing and potental world food market. And the fifth objective is to develop non-food products, such as energy and materials, based on agricultural production.

For example, food storage losses are high, but might be reduced by a small amount of research and demonstration. On quality, Europe could aim to make a smaller quantity of a better wine, or to make high-value cheese from milk rather than low-value powder. ("It makes me mad that we distil wine and dry milk", says Fasella.)

On alternatives, farmers could grow new crops, aided by a little research on what would grow where and be profitable. Fasella himself, before he joined the Commission from biotechnological research in Italy, had a hand in a successful project north of Rome. There, farmers agreed to change from the production of price-supported wheat to strawberries and flowers, with the help of a little science and low technology.

And on agricultural industry, Europe could, for example, re-examine the use of wood as a material and chemical feedstock. "Lignin [a major wood-waste] is still too little studied", Fasella says.

Exactly when these and related proposals will bear fruit in the form of research contracts with the Commission is not clear, but the ideas are now roughly at the stage of Esprit a year ago. Britain is pushing hard for reform of CAP. Most nations at least recognize its over-weight, and all might see the emerging research proposals as a useful aid to change — or to defusing a politically dangerous situation. If things moved as fast as Esprit, there could be tenders by 1984.

Besides agriculture, health is also on Commission minds. The cost of the European public health services (111,000 million European units of account a year — £61 million — for the 10 EEC countries except Greece, according to World Health Organization figures) could also be reduced by the right kind of research, Davignon and Fasella are arguing. The bulk of health service costs come in hospitalization, and only 14–15 per cent in drugs. The drug companies have had little motivation to develop preventive therapeutic measures — but the profitability of new drugs is declining rapidly. On both sides, therefore, and in other sectors of the health industry, there is an interest in a new approach. As in the agricultural sector, the Commission should start by an analysis of costs, Davignon and Fasella argue. From that point, financial priorities will determine research and development needs and ultimately new products.

Overly ambitious? "We are not aiming to be an eleventh state [beyond the ten of the EEC]", Fasella insisted last week. Rather, the Commission is determined to act as a strong service — to provide new, realistic means of action for member governments. And for the first time, it seems — if Davignon can convince his fellow commissioners — research and development are going to be serious tools of that action. **Robert Walgate**

Carter appointees leave NSF

Washington

Edward A. Knapp, the new director of the National Science Foundation (NSF), has arranged the departure of the three other past presidential appointees at NSF. The surprise move apparently came at the request of the White House which aims to install its own appointees and replace "holdovers" from the Carter Administration.

Donald N. Langenberg, deputy director of the nation's basic science agency, which is traditionally viewed as nonpolitical, will leave by the end of the year, at the request of Knapp and the White House. Langenberg, who is on leave from the University of Pennsylvania, had been a candidate for the director's job. The sudden departure took even NSF's governing board by surprise.

A second Carter appointee, Eloise E. Clark, deputy director for biological, behavioural and social sciences, has reportedly submitted a letter, stating her intention to resign, also at Knapp's urging.

Francis S. Johnson, deputy director for astronomical, atmospheric, earth and ocean sciences, also plans to go. His three-year leave of absence from the University of Texas at Dallas expires soon anyway.

The departures fuelled rumours that the Reagan Administration is politicizing the NSF. But a source close to the foundation said that "NSF is due for a surprisingly large increase in the fiscal 1984 budget, and Knapp was under pressure to have his own people there" in time for the announcement of the figure in January.

The chairman of the National Science Board, Dr Louis Bramscomb, said he had no information suggesting that the departures imply political influence but, he said, "that could show up if Ed Knapp is not given the independence to pick replacements with the highest professionalism. **Deborah Shapley**

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West German nuclear power

Fast breeder escapes veto

A vote in West Germany's federal parliament (*Bundestag*) last week promises to relieve pressures on the CDU/CSU/FDP coalition government by helping to remove the technical problems of atomic energy from political debate.

In last week's vote, the *Bundestag* finally agreed to lift the veto on the commissioning of the 800 MW fast breeder reactor at Kalkar. This veto was imposed in 1978 to prevent opponents in the SPD/FDP government from killing the project completely. A commission was appointed to inquire into the safety of the installation and the majority report, published last September, found that the risks of the sodium-cooled breeder at Kalkar were no greater than those of modern pressurized water reactors. The *Bundestag* thus had little choice but to permit start-up in 1983, although most of the SPD used the pretext of the minority opinion to vote against lifting the veto.

From its beginning in 1971, the entire project has been bedevilled by controversy. Delays over the siting of the reactor at Kalkar were followed by the parliamentary veto. The fast breeder project has also been a catalyst to the rise of the "Green" parties as a political force. Other, technical, problems have arisen over technology licensing and in proving the safety and containment in case of accident. According to the review carried out recently by management consultants A.T. Kearney and Motor Columbus for the Ministry of Research and Technology, mismanagement has also contributed to the increased costs and delays.

The overall result is that the costs for the project have been repeatedly revised upwards at a rate well above that of inflation, and now stand at DM 6,500 million (US $2,700 million), up by DM 1,500 million since 1980. Earlier this year, it looked as if this reactor and the other, high temperature, prototype reactor were running out of money. But the government has now found enough to keep them going for 1983, partly through cutting back other energy projects. As a result, well over 10 per cent of the 1983 research budget will be spent on the two prototype reactors.

Further development will depend on investment by the utility companies, based on the *Lander*. They will judge the reactors on the financial returns and it appears at present that the fast breeder will not be competitive before the end of the century. Thus there is a suspicion that a different standard is being applied to nuclear energy from that for other energy research.

On the other hand, the high temperature reactor may interest a wider spectrum of industry, although the prototype has also been subject to delays and increases in cost.

The main interest in Germany is in the use of high temperature steam for the gasification of lignite and bituminous coal, of which there are extensive reserves. Combined heat and power schemes, already in use in some northern cities with conventional power stations, provide an outlet for waste heat. Finally the reactor might be used to drive thermochemical cycles for splitting water to produce hydrogen. The EEC Commission has made hydrogen a major research subject and several cycles are being studied at the Joint Research Centres and elsewhere.

J.S.Dunnett

European atom treaty

New rules

Brussels

France's agreement with India to supply the Tarapur nuclear power station is being questioned by officials in Brussels. The agreement may be breaking the Euratom Supply Agency's monopoly over the supply of nuclear materials which has existed since the Euratom treaty was signed in 1958. The inquiry could be used as a way of applying pressure on France to agree to a revision of Chapter 6 of the treaty which the European Commission is proposing in order to bring it in line with the changes that have taken place in the nuclear industry since 1958.

The new proposals are the third attempt in eight years to bring the treaty up to date. In the past both France and the United Kingdom have opposed such a move in order to protect their nuclear fuel companies, British Nuclear Fuels Limited at Sellafield (Windscale) and Cogema at Cap de la Hague. The Commission suspects that both these companies have been using their market dominance to maximize profits by rigging prices and imposing unnecessary conditions on their customers in the rest of the European Community. Customers have been persuaded not to hold stocks of reprocessed fuels or to sell them to other users and the Commission feels that it would at once be realistic to abolish the illusion of a Euratom monopoly and to insist on a genuinely free market for the nuclear fuels industry. Otherwise there may be some risk that countries such as Belgium and West Germany may decide to build their own reprocessing plants, which would lead to over-capacity in Europe.

The new rules which the Commission is putting forward would give the European Commission the power to penalize anyone imposing any restrictions on trade in nuclear materials inside the Community. Exports, on the other hand, could be contracted on a bilateral basis provided the Commission can ensure that the contract would not damage the interests of any other EEC country. But a certain solidarity amongst the EEC member states would continue to be enforced in the event of a shortage when the European Commission would undertake to share out stocks equitably. The Euratom Supply Agency, which in the past has been successful in negotiating long-term uranium supply contracts with Australia, Canada and the United States, would continue to supervise and negotiate such deals. **Jasper Becker**

Stanford cuts comment on patent

Washington

Stanford University, citing "erroneous public impressions" concerning the Cohen-Boyer biotechnology patent, last week ordered the US Patent and Trademark Office to keep secret all future action on its pending application. The move came just before the patent office was expected to announce its latest action on the application, which it tentatively rejected last summer.

Normally, all actions on a patent application are secret until a patent is granted but Stanford originally waived its right to secrecy. According to Robert Rosenzweig, Stanford's vice-president for public affairs, the "central issue" in Stanford's decision to close the file was "the dispute over inventorship" (see *Nature* 25 November, p. 303). "We thought that had some potential for damaging people's reputations unnecessarily", he said. Dr Robert Helling, a co-author of the key scientific paper on which the patents are based, is now claiming that he is a co-inventor; the confusion over Helling's role was cited in the patent office's tentative rejection.

Rosenzweig said that keeping the file open had been "an experiment with some risk" and that "the experiment has failed". He said, "when the application process is completed, the file will be opened for public inspection. Until then, it is better that it not emerge in piecemeal fashion."

Stanford has already sold licences to the first Cohen-Boyer patent, issued in December 1980, to 73 companies for an initial fee of $10,000 per year. That patent, which covers the basic process for inserting foreign DNA into bacteria, is similar to the second one, now pending, which covers the transformed bacteria products. Thus there has been concern that the flaws the patent office cited in the second patent could invalidate the first patent. The licensees have discussed the possibility that Stanford should open the first patent for reexamination and meanwhile hold the licence fees in escrow. Rosenzweig said that the licensees would be kept informed of the progress of the second patent. **Stephen Budiansky**

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Chemical warfare

Fight amongst Finnish

The publication last week of the report of the United Nations on allegations that the Soviet Union and Vietnam have been using chemical warfare against insurgents in Afghanistan and South East Asia has caused some embarrassment to the Finnish foreign minister Paer Stenbaeck, who refused to let his ministry assist the enquiry. According to the independent newspaper *Laensi-Savo*, the minister was "mixing up the work of Finland's Technical Research Institute with our foreign policy's credibility".

The implication from comments in the Finnish media is that there has been a conflict over the freedom to research, with Mr Stenbaeck forbidding the project and the head of the Technical Research Institute (VTT) Dr Pekko Jauho insisting on the research going ahead.

During the past ten years, Finland has built up, under the supervision of the foreign ministry, a chemical warfare-monitoring capability which involves a number of laboratories and institutions — mainly civilian — including VTT. This capability, the Finns hope, will ultimately be used under the auspices of international agreements banning chemical warfare. Meanwhile the Finns have built up a considerable body of experience, and it was reasonable, after the pledges of successive Finnish governments to support the United Nations in every aspect of its peacekeeping role, that the UN enquiry team should approach the Finns for assistance in analysing vegetation and blood samples from South-East Asia.

But Mr Stenbaeck was reluctant to let his ministry help. He felt, apparently, that without the guaranteed conditions for impartial and neutral verification which would presumably be embodied in a future treaty, it could damage his ministry's credibility to be involved in such a contentious issue as the allegations of chemical warfare in South East Asia. In particular, he had considerable doubts about the provenance of the samples, which had been brought to Finland without prior notification or agreement. The sample delivered to the Finnish Foreign Ministry was accordingly returned to the United Nations unopened, apparently on Mr Stenbaeck's direct instructions.

The other two samples were delivered to two scientists working for VTT, Professor Tor-Magnus Enari, an expert on *Fusarium*, and Professor Rolf Rosenbere, a physicist working in neutron-activation analysis. VTT, a government establishment responsible to the ministry of trade and industry, operates a wide programme of contract research and development for both Finnish and foreign customers, and the UN commission was accepted as a purely commercial proposition. The work duly went ahead, giving, as it turned out, entirely negative results. **Vera Rich**

UK nuclear power

PWRs "safe"

A new report from the Advisory Committee on the Safety of Nuclear Installations (ACSNI) concludes that the Central Electricity Generating Board (CEGB) will be able to reach acceptable standards of safety for Britain's first pressurized water reactor (PWR), but draws attention to a number of areas in which it feels further work will be needed. In this respect it is substantially in agreement with the Nuclear Installations Inspectorate (NII) report earlier this year.

Some of the points raised by the document are sure to be used by objectors at the forthcoming public inquiry into the proposed PWR at Sizewell to embarrass CEGB, but the board seems confident that the questions raised in the ACSNI report have been answered since the end of 1981 (the latest date for ACSNI's evidence). This may explain some of the report's apparent ambiguities. In particular, CEGB's design proposals in its Preconstruction Safety Report have already been modified to include a double-containment system for the reactor pressure vessel, in accordance with current French and German practice.

ACSNI, set up to report to the Health and Safety Commission, attempts only to review the technical work of others and considers only selected aspects of PWR safety. The areas considered, by separate study groups, were the integrity of the pressure vessel, the plant/operator interface, and fuel processing. Most of the potential problem areas highlighted by ACSNI lie in the first two catagories. Besides echoing NII's insistence on the highest achievable quality control of steel components in the reactor vessel itself, the report asks for equivalent standards to be applied to the whole of the coolant system. Standards for testing the integrity of welds are questioned, and more work is requested on degraded core phenomena. One of ACSNI's most significant criticisms of the CEGB Preconstruction Safety Report concerns the possibility of multiple breaks in the steam generator tubes, a consideration apparently excluded by CEGB on the grounds of unlikelihood. Since that time such an accident has in fact happened, and resulted in the release of some radioactivity.

On the subject of the plant/operator interface (one of the main causes of the accident in the PWR at Three Mile Island) the report makes recommendations about management and training schemes, and

Japan plans DNA database

Washington

Japan's entry into the field of molecular genetics may include a central data bank for DNA sequences. Storage of these complex sequences in computers and their statistical comparison have opened up new analytical possibilities in the field.

Some Japanese scientists attending a recent workshop in Aspen, Colorado, expressed an interest in starting such a database, and the scientists who run the new US database at the Los Alamos National Laboratory have welcomed their interest. The Japanese Ministry of Education may decide on 7 January 1983 to fund the project.

The database, likely to be located at the University of Tokyo, could be linked electronically to the Los Alamos database and to that run by the European Molecular Biology Laboratory (EMBL) at Heidelberg, West Germany.

A crucial question is which sequences it should collect. It would clearly be useful to collect the sequences published — sometimes in Japanese — in the Japanese scientific literature, which are generally unavailable to the groups at Los Alamos and Heidelberg. On the other hand, the number of sequences published in the Japanese journals is so far rather small, so that the database may be expanded to include those published abroad as well.

The Los Alamos database is supposed to contain all published sequences by July 1983, according to the terms of the contract that the US National Institutes of Health awarded to Bolt, Beranek and Newman for the Los Alamos-based project, known as GenBank. Walter Goad, the principal investigator at Los Alamos, said last week that sequences with an estimated 750,000 bases had been entered into his system, and that by the July 1983 deadline, it may include as many as 2 million bases. The GenBank database has just been announced "as available to the public for a nominal fee". **Deborah Shapley** says that a simulator designed to train operators specifically in the control of PWRs should be operational before the reactor is commissioned. The CEGB is again happy that it can satisfy this request. The difficulty is, as the report points out, that no-one knows exactly how the reactor will behave in extreme fault conditions, so a simulation of such events can only be approximate.

NII has welcomed the ACSNI report as a valuable input to its own examination of the CEGB case, but says that all the areas mentioned by ACSNI are currently receiving attention anyway. The inaccessability of detailed current CEGB safety proposals is one major headache for objectors at the inquiry. **Tim Beardsley**

US energy research

Cuts demand

Washington

The Office of Management and Budget (OMB), still putting the final touches to the President's budget for fiscal year 1984, has proposed substantial cuts in research spending on fossil and solar energy and energy conservation. The OMB proposal, which is still subject to an appeal by the new Secretary of Energy, was leaked last week by congressional sources.

The proposed cuts are similar to those proffered by OMB for the past three years and which Congress has chosen to ignore. In addition to eliminating all demonstration projects and all federal government involvement in service programmes (for example grants to communities to provide insulation of residences), the cuts would hit the following research areas:

- Fossil energy. Research on combustion, coal liquefaction and gasification, and coal clean-up would be cut from the current level of approximately $300 million (as approved by the House and Senate appropriations committees for fiscal year 1983) to $106 million, the same level that the President requested for 1983. Most of these funds go to research contracts with industry.
- Solar energy. Long a target of Reagan Administration budget-cutters, solar research would also be held to the level of the 1983 request, $72 million. Congress is heading for an appropriation of $180 million for 1983. The Department of Energy itself had asked for $87 million for fiscal year 1984.
- Conservation and renewable energy. OMB wants a limit of $36 million on this programme which has already been severely cut by the removal of all non-research components. Of the $383 million appropriated by Congress for conservation in fiscal year 1982, $156 million was for research; the Department of Energy (DoE) requested $51 million for fiscal year 1984.

DoE is reported to be planning an appeal, at least for the fossil and conservation budgets. The final budget will be released in January. **Stephen Budiansky**

Soviet economy

Size costs dear

Last week, an article in *Pravda* by N. Kulagin, a hitherto obscure researcher at the N.A. Voznesenskii Financial-Economic Institute in Leningrad, evoked a flood of speculation in the West that, under its new leadership, the Soviet Union might reverse its traditional bias towards larger and more comprehensive industrial enterprises. Such an article is a standard method of launching a new policy trend, without going as far as to condemn the old.

In this case, under the title "It is hard to be universal", Kulagin compares the Soviet preference for "gigantic multiprofile plants" with the development in the West of small specialized factories which cooperate with major industry by providing specialized components and services.

The large Soviet enterprises, each with its own staff of repairmen, instrument makers and transport-workers, is, Kulagin pointed out, wasteful of resources — in the Soviet machine building industry, for example, some 38 per cent of staff are engaged in such ancillary work, compared with only 11 per cent in the United States.

The type of centralized planning practised in the Soviet Union, as well as the vast investment originally needed to bring a number of sectors up to world level, undoubtedly has had much to do with the preference for gigantic plants. Such factories produce not only the goods specified in the State Plan, but also a large part of the components and auxiliary equipment they require. As Kulagin points out, these cause considerable difficulties but do not show up in the total output figures of the plant, thus making nonsense of the economic indicators.

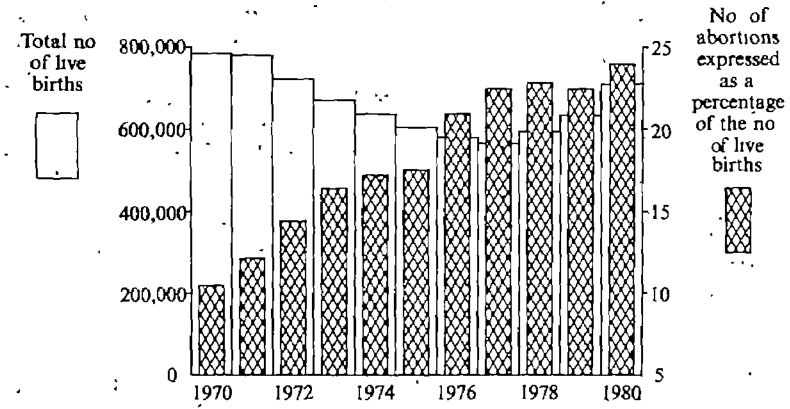
Furthermore (a point not developed by Kulagin) if a "giant" industrial plant in Leningrad needs say, bolts with special performance parameters, it will commission some Leningrad institute to do the appropriate research, while a plant in Vladivostok, needing a precisely similar bolt, will commission the appropriate institute there. The duplication of research is considerable, particularly because, officially, Soviet scientists are not supposed to discuss their unpublished results with colleagues from other institutes. The introduction of small-scale plants, each specializing in the production of specific components, as advocated by Kulagin, could cut research costs considerably.

The implementation of the latest results of research and development — the major bottleneck in the modernization of Soviet industry — could also be carried through more effectively, in a network of small-scale plants, since the individual shut-downs for retooling could be better staggered.

And, perhaps most important, according to Kulagin, the costs of components made in one's "own" factory are frequently double what they would be from a "specialized" factory.

The concept of a changeover to smaller production units is not — in spite of the commentators last week — entirely new. The trend has slowly been taking shape over the past ten years, and several planning conferences have been held on the theme, one of which proposed the formation of a special Ministry of Interbranch Production to run the small component-producing plants. This has so far, however, failed to materialize, owing to bureaucratic difficulties inherent in the transfer of plants from one ministry to another. **Vera Rich**

Increasing abortion rate in Britain



The annual number of abortions performed in England and Wales has risen from 82,730 in 1970 to 159,503 in 1980. In 1979 25 per cent of abortions were performed on women aged under 20 although only 9 per cent of all mothers fall inside this bracket. Not surprisingly, 60 per cent of the abortions were performed on women of between 20 and 34 — the age bracket which includes 85 per cent of mothers. The highest abortion rate is for women over 45 where the number of abortions actually exceeds the number of births. Source: *Health and Personal Social Services Statistics for England 1982* (HMSO: £7.50).

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UK engineering

Institutional scrap continues

Britain's 200,000 chartered engineers are currently voting in a postal ballot on a recommendation from the board of the Council of Engineering Institutions (CEI) that control of its register of accredited engineers should be handed over from its own Engineers' Registration Board to the new Engineering Council, set up in the wake of the Finniston report. The outcome of the vote is far from certain; however, it is clear that if the recommendation is approved, CEI, which represents members of all the chartered engineering institutions, will have little reason to continue its existence.

The objects of the Engineering Council, listed in its Royal Charter, are to "advance education in and promote the science and practice of engineering . . . for the public benefit". Its members, who are appointed initially by the Secretary of State for Industry, will form a number of standing committees. The main purpose of these committees is to consult with industrial companies and educational establishments as well the existing professional institutions.

Engineering Council on 30 September detailed its future intentions in all its areas of responsibility including the setting up of a register of engineers. The statement clearly was influenced by the previously expressed concern of CEI that the views of its members should be assured of an adequate hearing on the new council and that existing titles would be protected: CEI will ask the existing professional institutions to oversee the awarding of titles, and their independence is explicitly recognized.

However, the Engineering Council's document fails to satisfy the expressed wishes of CEI that the titles of Technician Engineer and Engineering Technician should be awarded only to members of institutions. Furthermore, the document fails to guarantee the CEI's wish for self-regulation for the profession. Despite this, the CEI board approved the move with a 25 to 10 vote, although some members apparently felt they had very little choice. Now a two-thirds majority in the postal ballot of members is needed to implement the transfer.

Mr Gerald Mortimer, CEI chairman, is satisfied that "somehow or other" the views of professional engineers will gain an adequate hearing in the new Engineering Council. The move has been welcomed by the Fellowship of Engineering, which points, out that "it will be essential for professional engineers to use their influence in the institutions to maintain standards".

Tim Beardsley

US research

Basic physics in doldrums

Washington

Basic physics research in the United States is declining in quality compared with Europe, according to a new study by the US Office of Naval Research (ONR). Moreover, US industry is "just not playing the game" in basic physics, with the exceptions of Bell Laboratories and the IBM Corporation. Finally, the study suggests that the US Department of Defense has supported less and less of the important contributions to basic physics in the past decade.

These are the gloomy findings of a study counting the affiliations of all the contributors to *Physical Review Letters* (PRL), a leading journal of research in basic physics, between 1958 and 1979. Author Ted G. Berlincourt of ONR and his colleagues also counted the affiliations of contributors to the leading journal for applied physics, *Applied Physics Letters* (APL), from the time it was founded in 1962 until 1979 and found the same trend.

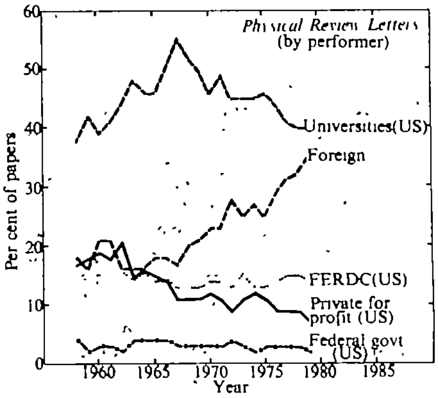
The accompanying figure shows the most striking finding of the PRL count. The journal published an increasing number of papers each year until 1968, when the number levelled off at approximately 1,000. Yet from the mid-1960s onward, the number of foreign contributors rose steadily and was still rising in 1979 when the count stopped. The number of US contributors to the journal decreased correspondingly.

The ONR study speculates that this dramatic shift in foreign contributions might be caused by editorial policy. But George Trigg, who has been editor of *Physical Review Letters* since 1966, says no decision was made either to take more foreign contributions or to level off at 1,000 papers per year. "It has always puzzled me, because in the last 15 years the numbers of articles in other journals has increased" Trigg said. Apparently, no more than 1,000 are good enough to get through PRL's review system. The probable conclusion is that fewer and fewer US papers have passed muster.

Institutions outside the United States whose scientists are most successful in publishing in PRL are, in order, the Kernforschungsanlage Jülich in West Germany, the Atomic Energy Agency of Canada, and IBM Switzerland.

Berlincourt and his co-authors see several alarming trends emerging from their study. The "vigor" shown by basic physics outside the United States, and the parallel "vigor" of applied physics, "will without doubt be translated into stiff competition in all technology-dependent international arenas".

The applied physics work of US industry, they note, is "near stagnation". Private companies in the United States have contributed a declining proportion of the papers in *Applied Physics Letters*. There has also been a decline in industry's contributions to *Physical Review Letters*; when Bell Laboratories' and IBM's contributions to PRL are taken out, industry has been contributing only 20 papers per year to PRL. IBM's and Bell Laboratories' contributions have declined since 1975.



From: "Performer affiliations and funding sources for research" by Ted G Berlincourt, Harold Weinstock, Francis Narin, and Samuel R. Reisher.

The paper also notes that the number of physicists supported by the Department of Defense (DoD) contributing to PRL, the basic journal, declined precipitously after 1967, the year when the Mansfield Amendment determined that DoD should support only research directly relevant to military requirements.

Although the Mansfield Amendment was in effect for only two years, the trend it accelerated "had not been reversed by 1979, some eight years after the expiration of the Mansfield Amendment", even though senior defence officials since the Ford Administration have been saying they wish to rebuild DoD's ties to the university basic science community.

"The very marked erosion of DoD support for PRL-type basic physics . . . implies a significant decoupling of the defence establishment from the cutting edge of the scientific discipline, which, probably more than any other, has yielded the seminal scientific advances on which revolutionary military development are based. . ." says the study.

The poor performance of industry and DoD in basic physics research may be correlated because each year DoD reimburses industry for some fraction of relevant research through the Independent Research and Development funds. So US industry has invested very little — even of its DoD money — in basic and applied physics. Overall, the study suggests that the recent rise in US defence research funds may not automatically reinvigorate US basic physics.

Deborah Shapley

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CORRESPONDENCE

Whose game?

Sir — In his review of J. Maynard Smith's *Evolution and the Theory of Games*, R.C. Lewontin[1] refers to his own "introduction in 1961 of the apparatus of game theory into evolutionary biology". Students of the history of ideas may be interested to know that, about a week after a seminar on this subject given here by Lewontin in 1962, and shortly before R.A. Fisher's death here, the latter also gave us a seminar on the same subject. With the "characteristic modesty" to which Lewontin also refers in his review, Fisher pointed out that not only had he introduced game theory ten years before von Neumann[2] but he had also discussed it in relation to evolution before Lewontin: this was in a paper that was given to the 30th session of the International Statistical Institute at Stockholm in 1957 and subsequently published twice[3,4]. I shall leave readers to judge for themselves the importance of these contributions but would like to record the main example that Fisher gave us at the seminar since I think it was never published and may be of widespread application to the explanation of some life cycles that are ecological enigmas. He suggested that the massive production of fruit which occurs infrequently and at random in northern deciduous forests ("mast years") illustrated the principle that, if an opponent (in this case nut-eating animals) cannot be defeated, then it is advantageous to randomize vulnerable activities (nut-production). His biographer[5] records Fisher's fondness for pigs and, since these animals used to be a major beneficiary of mast years, I suspect that there is a connection.

P.G. Martin

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On Time defended

Sir — I was interested to read the review by P.W. Atkins in the 11 November issue of *Nature* (p.133) criticizing *On Time* by Michael Shallis, since I have recently read and enjoyed the book. I found the tone of Atkins's remarks derogatory in the extreme.

A point that I would like to make is that scientists of the first category (as defined by Atkins) are so often the blinkered, passive donkeys of their professions who will deny a goodly portion of life's experiences because it is not possible to measure them quantitatively. I imagine that had Atkins been around at the time of Galileo he would have been enthusiastically pouring scorn on the "outrageous" heresy of a heliocentric planetary system for much the same reasons as he scorns Shallis's attempt to look at subject matter which is regarded as "unscientific". It is so easy to be an Atkins and to use these reductionist attitudes to stay safely within the comfortable known behaviour patterns of the peer group. It is very difficult to be a Shallis and to admit that we do not know it all and that there are areas where we can only wonder and conjecture.

I would like to know what indications Atkins has managed to find in the book that Shallis "disregards the successful continuing progress of modern science". It seems strange that Atkins should carp about this as his own popular lecture on all science rapidly coming to a single all-embracing conclusion, leaving scientists as "keepers of the truth", leaves one with the strong impression that here at least is one scientist who has come to a grinding halt and who one would expect to welcome, not deride, others whom he sees, however wrongly, as being in the same boat.

On Time is a well written, thought-provoking book which deserves better than to have attracted such a destructive onslaught.

V. Ann Buckle

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Cometary record

Sir — In my News and Views article on the recovery of Halley's Comet (*Nature* 25 November, p.318), I stated that Halley's Comet (at recovery 11.04 AU away from the Sun) was the most distant comet ever seen, beating the previous record held by Comet Stearn (1927IV). I was wrong and must thank Professor R.A. Lyttleton for pointing out my error. According to S.K. Vsekhsvyatskii (*Physical Characteristics of Comets*), 1927IV was last seen on 13 March 1931 when it was 11.53 AU from the Sun. So Stearn is still the record holder. For Halley to usurp its position we will have to observe it into August 1989.

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Grouping Darwin

Sir — In his autobiography *The Gates of Memory* Sir Geoffrey Keynes[1] revealed that the family he used to illustrate the inheritance of the ABO blood groups in his book *Blood Transfusion*[2] was his own and his wife's. Charles Darwin was Lady Keynes's grandfather.

Of Charles and Emma Darwin's five children, four were blood-group O and one, George, Lady Keynes's father, was untested. Charles and Emma must therefore each have carried at least one O gene, and of their grandchildren, only George's children could provide further relevant information, since George's blood-type was not determined.

George's wife Maud was blood-group A, and of their four children, two were O (one was Lady Keynes) and two were A. Maud must therefore have been of genotype AO, and George must have carried at least one O gene. In the published genealogy only the two "O" children themselves have children, who therefore provide no further information about George.

The possibilities are therefore that Charles and Emma Darwin might each have been AO, BO or OO, and so might their son George. For each of the nine possible genotype-pairs for Charles and Emma we can compute the probability that they had four OO children and a fifth who on marrying an AO had two O and two A children (and hence certainly carried on O gene).

The calculation of these probabilities is entirely straightforward and I therefore omit the details; it should be noted that because the only person other than Charles and Emma entering the genealogy except as a child is of known genotype (Maud, who is AO), the probabilities are not functions of the unknown gene frequencies in the general population. This is, of course, unusual in this type of problem.

The resulting probabilities expressed relatively are the likelihoods[3] of the several hypotheses:

Relative likelihoods of Charles and Emma Darwin's genotypes

OO × OO	16,384
OO × AO or AO × OO	800
OO × BO or BO × OO	544
AO × AO	43
BO × BO	19
AO × BO or BO × AO	26

It will be seen that it is overwhelmingly likely that both Charles and Emma Darwin were blood-group O, the next most likely hypotheses being over 20 times less likely.

A.W.F. Edwards

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Acidity and alkalinity

Sir — "Acidity" is an awkward term when used as a heading for discussion of effects brought about by varying hydrogen ion concentration. It is limiting in first appearances because it also doubles in general usage as a term implying only the acid end of the scale. The older term "hydrogen ion concentration" does not itself convey the concept we are often trying to put across; that which we neatly sum up in the jargon term "*p*H".

When we are heading manuscript sections for variables such as "temperature", "pressure" and so on, the heading word "*p*H" seems too short. We tend to get around the problem by writing "effect of *p*H". Some manoeuvring is also required to ensure that we do not have *p*H as the first word of a sentence, since we cannot capitalize it.

For the parallel concept of oxidation and reduction we rarely use the symbol "E", meaning the oxidation–reduction potential, in a sentence. Certainly the phrase "transferable electron potential" is an unusual heading. The convenient word "redox" has come into common use.

Therefore, by analogy, I propose the term "alkacidity" as a convenient word to express an important idea in brief.

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NEWS AND VIEWS

A superfast new pulsar

from David J. Helfand

FIFTEEN years ago last autumn, Morse-code-like signals pulsing with extreme regularity were received at a Cambridge radio telescope — pulsars had been discovered. Although the first such object was dubbed an LGM (for Little Green Man), a total of four such sources had been identified by the time the discovery was announced in February 1968 and the signals were soon explained, without recourse to the presence of extraterrestial intelligence, as lighthouse-like flashes from highly magnetized rotating neutron stars. With nearly 350 objects now catalogued, it might seem surprising that the detection of a new pulsar would generate much excitement. But PSR1937+214, whose discovery is reported by D. Backer *et al.* on page 615 of this issue of *Nature*, is not typical; it is rotating at an astonishing 642 times a second and may well represent the first of a whole new class of objects.

The story of the discovery is one of perseverance combined with a dash of good luck. The source first appeared in the fourth Cambridge catalogue of radio emitters as 4C21.53. Its peculiarity was first noted in 1972. Using the Cambridge interplanetary scintillation telescope with which pulsars were discovered, A. Readhead and A. Hewish reported[1] that the source 'twinkled' in the interplanetary medium, unusual behaviour for an object lying in the plane of the Galaxy where interstellar material generally broadens the apparent angular diameter of compact sources and destroys the IPS modulation. Backer's attention was drawn to the source in 1979 following a report[2] that it had a steeply rising spectrum at low radio frequencies which, along with the very small diameter implied by the IPS measurement, is a characteristic of radio pulsars. In perusing the literature, he noticed that 4C21.53 was roughly coincident with a diffuse region of emission with a contrasting flat radio spectrum. This combination of a scintillating, steep-spectrum source and a diffuse flat-spectrum region was strikingly reminiscent of the Crab Nebula and its embedded 33 ms pulsar, the youngest and fastest pulsar then known.

This area of the sky had been searched for pulsars in 1973 by R. Hulse and J. Taylor, then of the University of Massachusetts, using an extremely sensitive system at the Arecibo Observatory 1,000-foot radio telescope. As in most of the large-scale pulsar searches conducted to date, the workers would have found it impossible to detect a new pulsar with a period significantly shorter than that of the Crab since the threshold period for the search was ~ 30 ms. This constraint is usually dictated by limitation on computer time, but this had not been deemed a serious problem: there are no other objects like the Crab Nebula within the search range (~ 10^4 light yr), and it was thus assumed that there were no younger and faster pulsars to be found. The assumption, that all fast pulsars are young, may have been the critical flaw in the argument.

In 1979 Backer conducted a search for a shorter-period source in 4C21.53 at both the Owens Valley Radio Observatory and at Arecibo without success. His interest persisted, however, and, in November, some tantalizing earlier results from S. Kulkarni and C. Heiles were followed up with a search for interstellar scintillation which has, to date, only been observed for pulsars. Deep modulation was found and the following day, the periodicity of 1.5578 ms was confirmed.

The source is remarkable in many ways. The rotation rate is within a factor of about 3 of centrifugal break-up. The star's surface is moving at ~ 20 per cent of the speed of light and the rotational kinetic energy of the object is nearly 10^{52} ergs, comparable with the entire energy output of a supernova explosion. Furthermore, this superfast pulsar shows no evidence of the encircling supernova remnant expected to accompany the creation of such an object.

A measurement of the pulsar's slowdown rate is critical in addressing several important questions including the object's age, origin and energy loss mechanism (for example, gravitational waves versus magnetic dipole radiation and energetic particles). The limit quoted by Backer *et al.* implies a period derivative $\dot{P}$ $<10^{-15}$ seconds per second (s s^{-1}), a factor of several hundred smaller than the Crab. J. Taylor of Princeton University has recently taken his equipment used in monitoring the binary pulsar to Arecibo and has already lowered this limit substantially in a week's observation. It thus appears that, despite its huge store of rotational kinetic energy, the pulsars energy output is quite small. Optical observations, also reported in this issue, and X-ray data taken with the Einstein Observatory by R. Becker suggest that the high-energy emission from this object both from within the magnetospheric (that is, pulsed) and from a pulsar-powered synchrotron nebula is several orders of magnitude less than that from the much slower Crab pulsar. These results will dampen the initial excitement caused by the discovery among the experimental general relativity community, where the early report[3] of a high spindown rate led to quickly drawn plans for gravity wave detectors tuned to the pulsar frequency.

Backer *et al.* suggest that the pulsar and the nearby diffuse source (now known to be an HII region) may be associated, with the latter excited by an escaped binary companion of the fast pulsar. From the separation of the two sources and using a typical pulsar velocity, they infer an age for the pulsar of ~10^4 yr. The case is far from compelling, however. More recently, A. Alpar, M. Ruderman and J. Shaham of Columbia University, together with A. Cheng from Rutgers University, have proposed a scenario in which the neutron star is billions of years old and was reactivated as a pulsar after having been spun up by accretion from a companion. In a letter to appear in the next issue of *Nature* they suggest that the source is the remnant of a galactic bulge X-ray binary system. The standard scenario for bulge sources consists of an evolving low-mass star spilling matter onto a neutron star with a very low magnetic field. For such an object, the Alfven radius at which the magnetic field dominates the inflowing matter is very close to the stellar surface, allowing for a large transfer of angular momentum to the star, spinning it up to millisecond periods. The ultimate spin rate is a function principally of the field strength and the mass transfer rate. Once the accretion stops as a result of the dissipation or ejection of the companion, the pulsar will begin to spin down again. But with its low field, the spindown time will be very long, preserving a population of old rapidly spinning pulsars.

The strength of this picture is that it offers a prediction of the current magnetic field strength and, thus, of the period derivative for such a source. The model gives the right answer for $\dot{P}$ for each of the three known binary pulsars, all of which have anomalously low spindown rates for their short periods. For 4C21.53, the prediction of $\dot{P} < 5\times10^{-20}$ s s^{-1} will be testable within a few months.

Many questions remain to be answered about this exciting new object and searches for other examples are already being planned. Future studies will focus on what such sources can tell us about the mechanism of pulse emission, neutron star interiors, and the origin and evolution of pulsars. At the moment, though, 4C21.53 serves as a reminder of the richness of the physical Universe in which each unexplored corner of parameter space holds a new mystery for the astrophysicist to ponder. □

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'Lucy' jilted?

from M.H. Day

WHAT'S a girl to do if her date lets her down? This question often has to be answered by girls who have waited in vain — but the question of interest here is has 'Lucy' (or to be more precise her slightly older 'relations') waited 3.3 or 3.6 million years before her date with Don Johanson, Yves Coppens and Maurice Taieb? These three, the leaders of the French/American expedition to the Hadar region of Ethiopia, have been responsible for the recovery of the early hominid material, known collectively as *Australopithecus afarensis*, from deposits that were originally said to range in age from 2.6 to 3.1 million years before the present. The famous Lucy is said to be part of that species, as are Mary Leakey's earlier Laetoli hominids from Tanzania[1]. Earlier this year, however, it was suggested that the dates of the basalts from the Hadar series had been underestimated by as much as 300,000–600,000 years, bringing the earliest date of the Hadar site much closer to that of Laetoli, the site from which the *A. afarensis* type specimen was chosen[2], this species being regarded by some as representing the common ancestor of the hominid stem.

An article[3] in this issue of *Nature* (p.631) now questions this revision of the lower end of the temporal range of the Hadar hominids on grounds of stratigraphical correlation between the Hadar Formation and sites in the Lake Turkana Basin, together with faunal studies that may be consistent with the younger rather than the older age for the bottom of the Hadar sequence. F.H. Brown reports the results of microprobe and X-ray fluorescence analysis of a group of volcanic tuffs from the Koobi Fora Formation (Tulu Bor), the Shungura Formation (Tuff B) and the Usno Formation (Tuff U-10). All these air fall tuffs are the result of volcanic eruptions and all are found in the Lake Turkana Basin of northern Kenya and southern Ethiopia. Further north in Ethiopia, the Sidi Hakoma Tuff has also been sampled from the Hadar site and added to the comparisons. The microprobe analyses of major and minor elements and the X-ray fluorescence analyses of minor and trace elements show remarkable correspondence in all of the tuffs examined and lead to the view that all were the result of broadly contemporaneous events and thus may indicate isochronous litho-stratigraphical horizons. This is said to produce a 'direct lithostratigraphical tie' between the Hadar Formation and the Lake Turkana region which can help to date the fossil faunas of the two areas, including the hominids.

The palaeomagnetic evidence shows that the three tuffs from the Lake Turkana basin are all of normal polarity and they are thought to lie in the Gauss Normal Epoch, having therefore an age of between 3.17 and 3.42 million years. The Sidi Hakoma Tuff also has a normal polarity. The two reversed zones above the Sidi Hakoma Tuff and their equivalents in the Shungura and Usno Formations have been regarded as the Mammoth and Kaena Events.

Potassium/argon (K/Ar) and fission track dates from the Hadar Formation are said to be consistent with those identifications with one interesting exception, the 3.6 Myr date for the Type B sample of the Kadada Moumou Basalt (KMB)[2]. The date for this layer has been revised by Brown[3] to 3.12 Myr by applying a 3.5 per cent upward 'correction' to the 3.01 Myr date for the original Type A sample of the KMB, so that the reversed polarity of the KMB layer can sit conveniently in the Mammoth Event rather than be attributed to the underlying Gilbert reversal as it would be at 3.6 Myr. In the Lake Turkana Basin the palaeomagnetic identifications also apparently accord with the K/Ar dates on the tuffs, with the exception of the date of Tuff B10 of the Shungura Formation which is said to be 'too old' at 3.75 Myr. This tuff is one of the uppermost units of the Shungura Formation and lies just below Unit B10, which produced most of the fauna from Member B referred to in a second paper[4] published in this issue of *Nature* (p.633) that deals with the age of the Hadar Formation.

In these days of 'high-tech' science, there is something delightfully anachronistic about consulting an assemblage of fossil bones to support or disprove a numerical age produced by radiometric means. This was exactly what happened at Koobi Fora, however, where it took the 'pig clock'[5,6] to sort out the correct date for the KBS Tuff. Biostratigraphy, therefore, still has an important part to play in geochronology, since its cumulative effect may also be enhanced by comparisons from several adjacent sites. In the paper by Boaz, Howell and McCrossin (p.633), two large-mammal faunal lists, one from the Usno Formation (Lower B) and the other from Shungura (B10), have been compared with the Hadar assemblage and with each other by means of faunal similarity coefficients.

The Usno and Shungura comparative assemblages are given 'best-fit' K/Ar and palaeomagnetic dates of 2.92 and 3.15 Myr respectively and thus a time difference of about 0.2 Myr. The results show firstly that the Usno and Shungura assemblages are nearer to each other than either is to Hadar and secondly that by any test the Hadar and Usno faunas are closer than the Hadar and Shungura B10 faunas, suggesting that the Hadar fauna is closer to the older of the two dates. Unfortunately, sites such as Laetoli, Kanapoi, Mursi and Ekora, with a range of 3.2–4.0 Myr, cannot be compared with Hadar at present since their inventories are not complete. The faunal evidence, as far as it goes, therefore, seems to be consistent with the date for the Hadar site put forward by Brown, yet a pre-Usno date for Hadar is prudently not rejected. It seems that the correlative stratigraphical and faunal case for a younger lower limit for the Hadar site is interesting but not compelling.

What, then, would be the significance of this proposed revision (or perhaps reversion is a more appropriate word) of the dating of the Hadar site for hominid evolution? All seem agreed that the bulk of the Hadar hominids are older than 2.88 Myr. The lower limit of the range at Hadar is either 3.12 or > 3.6 Myr, a difference of 400,000 years or more with a possible span of 240,000–720,000 years at this site. This means that the Hadar sample of hominids is drawn from either a quarter or nearly three quarters of a million years; this must colour the views of those who would tackle the question of whether the Hadar sample is homogeneous and monospecific. Indeed, in relation to the Laetoli site, a gap of 370,000 years has re-opened in the evolutionary history of *A. afarensis*.

As well as the correctness of including the Laetoli hominid material in the same specific category as that of the Hadar sample, the question of the age of the Makapansgat site in the Republic of South Africa now begins to become relevant. A younger date for Hadar, if proven, coupled with an older date for Makapansgat[7,8], could make it uncertain that Makapansgat *Australopithecus africanus* is more recent than Hadar *Australopithecus afarensis*. Laetoli is, of course, still older than either site; but if the specific unity of the Hadar *A. afarensis* sample or the inclusion of the Laetoli hominids in that sample is shown to be incorrect, then *A. afarensis* could be a stem form in name alone. The folly of selecting a type specimen both geographically and temporally separated from the largest and best fossil sample would then be exposed once more.

To let a girl down on her date is bad enough, but to get her name wrong when you do arrive would be intolerable! ☐

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CORRIGENDUM

In the *News and Views* article 'Human monoclonal antibodies' (*Nature* 300, 316; 1982) D. Crawford's affiliation was given as the Imperial Cancer Research Fund Laboratories, London. She is in fact at the Department of Haematology, University College London.

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Mouse and supermouse

from Jeffrey G. Williams

THE mice displayed on the cover of this week's *Nature* are littermates but one has a bodyweight almost twice that of its sibling. They differ in that the larger mouse carries a hybrid gene containing the promoter of the mouse metallothionein-1 gene fused to the structural gene for rat growth hormone (see p.611). The 'transgenic' mouse was produced by injecting the hybrid gene into the pronucleus of a fertilized egg. Mice carrying the gene display very high levels of the fusion mRNA in their liver, high levels of growth hormone in their serum and an elevated growth rate. This is the first time that genetic engineering has been used to alter the phenotype of an animal in such a profound manner and is the culmination of a series of elegant experiments performed by Palmiter, Brinster and their colleagues over the past few years. There are almost certain to be great scientific and practical benefits from the application of genetic engineering techniques to animals. It is essential, however, to stress that there are limitations on what can be done — and more important, on what is worth doing.

When cloned genes are introduced into fertilized eggs by micro-injection a proportion of the mice that develop will carry the gene. The injected genes become chromosomally integrated but they are not found at the normal, nor even at a unique, location and the number of gene copies per cell also varies from animal to animal. The mice that develop from the injected eggs may also be chimaeras in which only some of the cells contain foreign DNA. However, a proportion of mice do transmit the newly acquired gene to their progeny and it s thus possible to produce mice containing the gene at a defined position in every cell.

The product of the mouse metallothionein-1 gene is a small cysteine rich polypeptide that binds heavy metals and is thought to be involved in zinc homeostasis and resistance to heavy metal toxicity. It is present in most tissues of the mouse, but the highest levels are found in liver. Transcription is inducible by heavy metals and glucocorticoids. Experiments reported earlier this year (Brinster *et al. Nature* **296**, 39; 1982) used a hybrid gene to establish that the sequence elements required for heavy metal inducibility lie within a stretch of DNA ninety nucleotides upstream of the start point of transcription. The hybrid gene contains a segment of the 5′ non-coding region and upstream sequences derived from the metallothionein-1 gene. Both were fused into the 5′ non-coding region of the structural gene for herpes virus thymidine kinase to provide a readily assayable enzymatic activity. The hybrid gene was injected into fertilized mouse eggs which were incubated with cadmium as an inducer and then assayed for thymidine kinase activity.

The same fusion gene was used in two subsequent studies in which micro-injected fertilized eggs were re-implanted into foster mothers (Brinster *et al. Cell* **27**, 223; 1981; Palmiter *et al. Cell* **29**, 701; 1982). A high proportion of mice which carried the fusion gene expressed thymidine kinase in the liver. The activity was inducible with heavy metals but not with glucocorticoid hormones. For unknown reasons, in the progeny derived from these mice the expression of thymidine kinase was diminished or abolished in some cases and enhanced relative to the parent in others.

The metallothionein-growth hormone fusion gene was constructed using exactly the same principle as the *tk* fusion gene, with the genes being joined in their 5′ non-coding regions. The mice contained variable numbers of copies of the gene and, with only one exception, the mice with the highest growth rate had the most copies of the gene. The mice were fed zinc to induce transcription of the fusion gene but this may not have been necessary. Most of the mice containing the gene were larger than normal before being fed zinc, and one animal which was removed from the zinc diet continued to grow at an accelerated rate. In mice with the highest copy number of the gene the level of growth hormone production was between 100 and 800 times the normal level. The serum concentration in one of the mice actually exceeded 100 $\mu g\ ml^{-1}$.

Thus, aside from the variability of expression in second generation mice and the lack of response to glucocorticoids, results with the metallothionein promoter are highly encouraging. Sadly for the developmental biologist, however, this is not the case for globin genes. Although transcripts have been detected in muscle tissue from one mouse line carrying the rabbit β-globin gene, neither the rabbit β-globin gene, nor a fusion gene containing the 5′ half of the mouse β-globin gene and the 3′ half of a human β-globin gene are expressed in the blood cells of seven mouse lines carrying the rabbit gene and two lines carrying the fusion gene (Frank Constantini and Elizabeth Lacy, personal communication). This difference in behaviour of the β-globin genes and the metallothionein gene in transgenic mice may be a reflection of a fundamental difference between the two genes. Globin genes are expressed only in cells of the erythroid lineage and only at a specific stage in development. While expression of the metallothionein-1 gene varies from tissue to tissue, most tissues express the gene at some level. Thus metallothionein might almost be regarded as a housekeeping gene which displays tissue specific variation in the extent of expression. This in no way detracts from the usefulness of this promoter in obtaining high levels of expression of any gene to which it is suitably fused, and this is powerfully demonstrated by the growth hormone study.

The authors discuss a number of implications of their results. The elevation of serum protein levels by these means could provide a way of studying hormone action a way of accelerating animal growth, a method of producing valuable gene products and a means of correcting genetic disease. Before considering these last three applications individually there is one point which should be made. They all rely to a greater or lesser extent on the transmission through the germ line of the ability to express the gene. However, the metallothionein-1 thymidine kinase fusion gene was sometimes not expressed in the offspring of mice which were themselves expressing the gene. If the metallothionein-growth hormone fusion gene behaves similarly this phenomenon will require further investigation.

The benefits which might accrue from a shorter production time seem a sufficient spur to ensure that fusion genes of this kind will be used to accelerate the growth of livestock animals. Also, the novel concept of genetic farming — the use of animals to produce large amounts of commercially important proteins — may be applied to the production of proteins which require secondary modifications not correctly made in yeast or other eukaryotic host cells. Finally, this achievement once again brings into question the potential of this technology as a means of correcting genetic disease. It will have become clear that such an approach will only be applicable where a genetic disorder may be prevented by simply elevating the serum concentration of a particular protein. In order to correct a genetic disorder such as a thalassemia by manipulation of the embryo, expression of the introduced gene would probably have to be limited to cells of the erythroid lineage and this is not at present achievable. Also, for reasons which have been presented in a recent review in this journal (Williamson, R. *Nature* **298**, 416; 1982) it seems exceedingly unlikely that there will ever be a clinical requirement for such therapy to be applied to the embryo. Control sequences such as the metallothionein promoter may, however, prove useful in developing methods of correcting gene disorders in the somatic cells of affected patients.

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Hypertension and inhibition of the sodium pump: a strong link but in which chain?

from Ian M. Glynn and T. J. Rink

THE experiments reported in this issue of *Nature* (p.650) by Hamlyn and his colleagues[1] from Baltimore, showing that the blood from patients with high blood pressure contains an inhibitor of $(Na^+ + K^+)$ATPase, are welcome for their cogency. Similar claims have been made before[2,3]; but always on the basis of evidence too indirect to be wholly convincing in a field where direct testing is feasible. The direct test has now been made and, though the effects are small, the answer seems clear. The blood plasma of patients with essential hypertension, that is, with a permanently raised arterial blood pressure not secondary to any known cause, is more effective at inhibiting a partially purified $(Na^+ + K^+)$ ATPase (the enzyme that pumps sodium ions across cell membranes in exchange for potassium ions) from dog kidney than is plasma from normal subjects; and the higher the blood pressure the more inhibitory the plasma tends to be. Furthermore, since plasma from most normal subjects is slightly inhibitory, the inhibitor may be of physiological as well as pathological interest.

The crucial question is whether correlation implies causation: does the inhibitor have a role in the aetiology of essential hypertension, and, if it has, what is that role?

There is no doubt that the new results fit well with the notion that essential hypertension is a disease in which the inability of the kidneys to cope normally with salt and water loads is compensated for, not only by a rise in blood pressure[4–6], but also by an increase in the plasma concentration of a (hypothetical) hormone that promotes salt and water excretion by inhibiting sodium reabsorption in the kidney tubules[7–9]. The circulating $(Na^+ + K^+)$ATPase inhibitor could well serve as the hypothetical hormone, though there is so far no evidence that it does in fact inhibit sodium reabsorption in the kidney tubules or that it has a natriuretic (promoting salt excretion) effect.

The results also fit well with the hypothesis that the narrowing of the arterioles — which everyone agrees is the immediate cause of the raised arterial blood pressure in established essential hypertension — represents a secondary effect of the increased level of the hypothetical sodium-transport-inhibiting hormone. The suggestion is that inhibition of the membrane sodium pump in the arteriolar smooth muscle cells leads to a rise in intracellular sodium concentration, which, by the action of a Na^+-Ca^{2+} exchange mechanism, increases intracellular calcium concentration and hence contractility[10].

Attractive though this hypothesis may be, it is still only a hypothesis. There is evidence that inhibition of the sodium pump in smooth muscle, by a reduction in extracellular potassium or by high concentrations of cardiac glycosides, can lead to an increase in arteriolar resistance in the rat[11], but it is not clear that the circulating inhibitor reported by Hamlyn *et al.* would have a similar effect, or, if it had, that the effect would be maintained. It is perhaps ominous that an almost identical hypothesis[12,13] proposed in the late 1960s to explain the ability of cardiac glycosides to increase the force of contraction of the heart still remains controversial. And though it is comforting to have a plausible hormonal explanation of the paradox that salt and water retention can lead to hypertension associated with a normal cardiac output and increased arteriolar resistance, the explanation proposed may not be sufficient or even correct. The consensus of opinion seems to be that in patients with coarctation of the aorta (a pathological narrowing of the aorta above the level of the renal arteries), the arterial blood pressure is high in regions above the coarctation and roughly normal in regions below it — in other words the pressure is adjusted to suit the kidneys — yet the blood flow through the arterioles is normal in both regions[4,6,14]. This implies that the calibre of the arterioles is reduced in the upper half of the body but not in the lower, a fact that is not easily explained if the reduced calibre of the arterioles is caused by the action of a circulating agent on the smooth muscle of their walls.

Strong evidence that a circulating agent can be involved in the genesis of hypertension comes from experiments in which rats of different strains had their skin and subcutaneous tissue joined so that substances could exchange between the bloodstreams. When rats from a strain that develop hypertension on a high-salt diet had their circulation linked to rats that did not respond in this way, the originally unresponsive rats rapidly developed hypertension when fed salt[15]. These experiments have been interpreted to suggest that the salt-sensitive rat on a high-salt diet produces a circulating natriuretic hormone which increases the contractility of the smooth muscle of the arterioles[9]. That is not the only possible interpretation, however; it is at least as likely that the effect of the transferred circulating factor is to make the kidney of the originally salt-resistant rat less able to excrete salt and water, and that the hypertension arises secondarily. It would be interesting to see whether plasma from the salt-fed, salt-sensitive rat behaves like plasma from hypertensive patients in inhibiting $(Na^+ + K^+)$ATPase.

Other questions that must now be asked concern the relation between the $(Na^+ + K^+)$ATPase inhibitor demonstrated by Hamlyn *et al.* and (1) the substances present in the plasma of hypertensive patients that can increase the vascular reactivity of rats to noradrenaline and angiotensin[16], (2) the high- and low-molecular weight natriuretic substances that have been extracted from the urine of salt-loaded subjects[17], (3) the natriuretic substance found in the urine of normal men immersed up to the neck in water at 34.5°C (ref. 18), and (4) the natriuretic and $(Na^+ + K^+)$ATPase-inhibiting substance found in the plasma of volume-expanded rats[19]. It will also be necessary to consider to what extent the presence of circulating $(Na^+ + K^+)$ATPase inhibitor can explain the numerous and sometimes contradictory reports of abnormalities in sodium, potassium and lithium transport in the red blood cells and white blood cells of patients with hypertension and of their relatives with normal blood pressure.

Presumably the first priority of the group at Baltimore will be to characterize their inhibitor. They have already shown that it is heat-stable, that it does not cross-react with antisera to cardiac glycosides and that it acts rapidly even at concentrations at which the maximal effect is small. It should not be difficult to gain a rough idea of its molecular weight, or to determine whether or not it is organic. More precise identification will be more difficult, but it must be an advantage that the assay is so simple. When the inhibitor is identified and can be prepared in quantity, it should be possible to determine whether it can produce a maintained narrowing of

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arterioles, whether it can promote salt excretion by the kidney and whether the kidneys of hypertensive animals or patients are less sensitive to it than are the kidneys of normal animals or patients. The answers to these questions should define the role of the inhibitor in the aetiology of hypertension — if indeed it has one. □

The prehistoric salt trade in Europe

from John Alexander

SUFFICIENT archaeological evidence has now accumulated for a picture of the winning and trading of common salt in prehistoric Europe to emerge. North of the Alps several big inland deposits were exploited and traded over long distances in the years before 500 BC. In the Mediterrean coastlands sea-salt was used, but further north, where boiling was necessary, this was not at first common. After 500 BC the inland suppliers disappeared and production moved to the coasts, perhaps as a result of the changes brought about by the mass migration of peoples at that time.

Common salt is not just a luxury for human communities with largely cereal diets, but is an essential. The salt received from flesh, blood and urine is insufficient for dietary needs. Salt may also be required for preserving flesh (including fish) and hides, or for religious purposes. Since substantial deposits of salt are rare in Europe and many regions are remote from seashores, salt was traded over long distances, as is known from the earliest written records (the last few centuries BC). Detailed knowledge of the industry in earlier prehistoric times has always been difficult to come by since the traded salt does not survive, but much recent excavation at the production centres makes it possible to find out when they were exploited, whilst a study of traded metal objects suggests the routes used for salt-trading[1].

Common salt is obtainable in quantity from four kinds of source — sea-salt, rock salt, saline springs and salt-rich plants — all of which were present and utilized in Europe. Sea (or bay) salt is only won with ease on flat coasts where advantage can be taken of tidal variation. In the Mediterranean coastlands the work is relatively easy as solar heat is sufficient to evaporate the brine. Elsewhere boiling is essential and the collection of fuel and the making of vessels are major additions to the work. The debris from the boiling process (broken vessels, burnt earth and charcoal) can be recognized and dated, as in the 'red hills' of Essex.

Rock (or haline) salt can only be obtained in quantity by mining, but needs little further processing. In the few localities where mining occurred, as in central Austria, shafts and galleries dug along the seams have been found and dated.

Brine from inland saline springs, or from the salt-impregnated earth around them (from which the salt must be dissolved and cleansed before boiling) will leave debris similar to the seaside boiling-sites and can, as in the Saale Basin in Germany, be recognized and dated.

Salt-rich plants can be burnt and their salt dissolved and evaporated by boiling. Tacitus reported the existence of the industry from Germany in the first century AD but it has not yet been recognized archaeologically although it would have left debris.

Before 500 BC coastal working of salt in regions where boiling was necessary (the Atlantic and North Seaboards) was rare[2,3] and only around the Mediterranean Sea, where solar heat was sufficient to evaporate the brine, does sea-salt seem to have been important[4]. Routes inland, such as the 'Via Salaria' in Tuscany (from near Ostia to the Sabine Hills), existed from at least the fifth century BC, but there is no evidence that sea-salt was carried up into central Europe.

North of the Alps, some inland sources of salt were used in the early part of the first millennium, as they had been in the second millennium, and the three areas with major reserves of salt, shown in the map below left, were increasingly exploited. Estimates of salt production show that it was in excess of local needs and that wide regions were being supplied[3,5].

In Austria, where names like HALLstatt and HALLein-Dürnberg preserve references to salt, thick veins of common salt were followed down for more than 400 m by skillfully constructed shafts and galleries. These, similar to contemporary Alpine coppermines, must have been the work of experienced miners[6,7]. They were especially active between 1000 and 400 BC when the 'eastern workings' produced thousands of kilogrammes of salt in heart-shaped slabs weighing up to 40 kg each. Nearby contemporary cemeteries are very rich in metal objects, especially jewellery, of types found within 200 km of the production sites and may well reflect the wealth resulting from a long-distance trade in salt slabs. The highly skilled miners appear to have worked only in the summer in the high, isolated alpine valleys at Hallstatt, and caravans of pack animals or porters would have been necessary to carry away the slabs. The mines appear to have gone out of use after 400 BC. A distant analogy with the Saharan fourteenth to sixteenth century AD saltmines at Taghaza,

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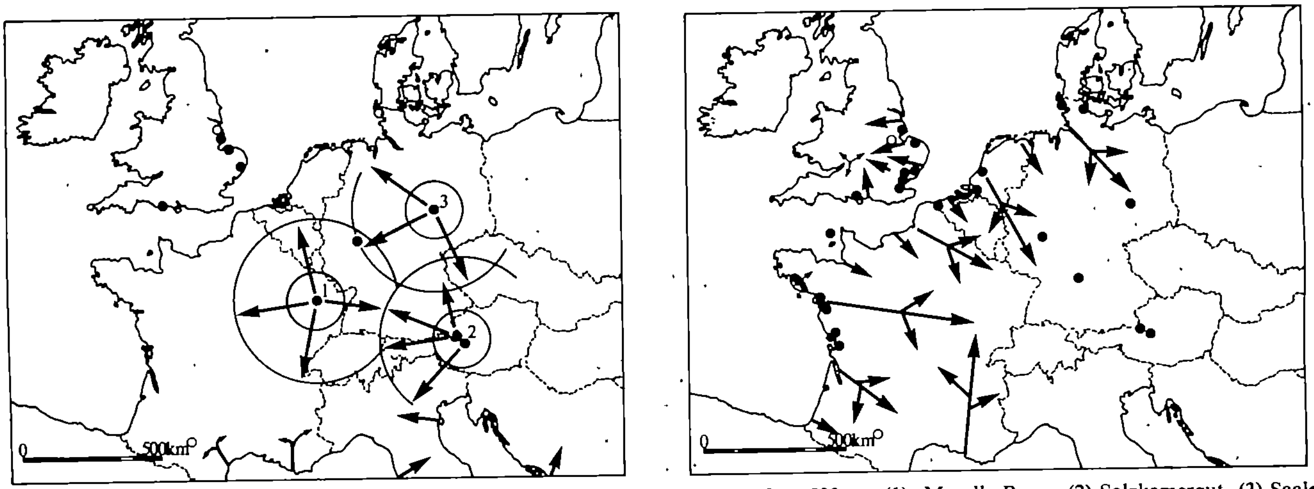

The suggested pattern of (left) salt production in western and central Europe before 500 BC: (1) Moselle Basin, (2) Salzkamergut, (3) Saale Basin; and (right) after 500 BC (after ref. 2).

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where the slabs were carried over 1,000 km by regular caravans, is interesting[8,9].

At the same time a different kind of large-scale exploitation was being practised in the Saale Basin in Saxony. Here around HALLe, salt springs over some 200 km^2 were exploited. The brine was boiled in batteries of distinctive pottery vessels and mounds of debris five metres high have been found. As in Austria, there is an absence of remains after 400 BC, suggesting that production ceased for some centuries[10,11]. Very large numbers of conical lumps of hard salt weighing several kilogrammes were produced and were probably dispersed over a wide area, perhaps several thousand square kilometres. The mechanism for production and dispersal may well have differed from that in Austria, since no great expertise would have been necessary, although the labour required suggests considerable organization. A distant analogy may be seen in the brine springs at Uvinza in Tanzania which are scattered over 30 km^2 and were worked seasonally by up to 20,000 people in the nineteenth century AD[12].

A third centre, worked on a similarly large scale in the same centuries, lay in eastern France among the brine springs of the Moselle valley near Seille. Here, over some 200 hectares, debris from brine boiling in great banks up to 17 m high are found. Once again production seems to have stopped after 400 BC. Production on this scale suggests that the salt served the needs of a wide area[13,14].

No other inland centres as large as these three have been found and it seems likely, in the absence of extensive coastal salt-boilings, that they supplied most of the North European Plain with salt (see map on left). Long-distance trading, as in Africa, was accompanied by trade in more durable, especially metal, objects[20].

A major change in salt production seems to have taken place after 400 BC. Large-scale working at inland centres stopped while the number of coastal brine-boiling sites greatly increased. The new sites appeared on the Atlantic coasts of France[15,16], Belgium[19] and southern England[17] in numbers too great merely to satisfy the needs of coastal communities (see map on bottom right of p.577). There was probably a salt-trade inland, by boat up the rivers, since there are many boiling-sites near estuaries. Trading of this kind penetrated some 300 km into the hinterland of West Africa in the nineteenth century AD[18].

That there was some connection between the decline of the inland centres and the rise of the coastal ones in the fourth to second centuries BC seems likely, but what brought about the change is not yet clear. Those centuries saw great changes in central Europe, with the migration of large numbers of Celtic-speaking peoples into Italy and the Balkans and the development of the La Tène culture. These may well have interrupted patterns of control and trade and been coupled with the exhaustion of supplies of firewood and the need for deep, easily flooded mines. Coastal production in areas with greater resources of firewood and with less opportunity for centralized controls may have taken advantage of this.

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Ball lightning wild and wonderful

from Martin A. Uman

BURBIDGE and Robertson report in this issue of *Nature* (p.623) the unusual observation of a liquid-like 'blob' entering a house under a door and moving along the floor immediately after lightning struck extremely close by. Because of the association with lightning, it is tempting to classify the 'blob' as ball lightning, although it represents an extremum in the spectrum of observations of that phenomenon for it had a very low luminosity, lacked a well defined shape and was not free-floating.

Reports of ball lightning can be found in both ancient and modern literature and the similarity in described size, colour, smell, duration, sound at demise and physical damage lends overwhelming support to the view that ball lightning is a real phenomenon (rather than a visual after-image or a psychological aberration). Nevertheless, no adequate laboratory simulation of ball lightning has been achieved, although similar phenomena are occasionally generated by accident in high-current electric machinery.

Most ball lightnings are spherical or oval, between the size of a marble and a basketball, exhibit a faint glow and last a few seconds. They appear to float while moving horizontally and can occur inside houses and all-metal airplanes as well as out-of-doors. There may well be more than one kind of ball lightning. For example, the bright spheres which hang high in the air and are seen at considerable distances would seem different from the typical ball lightning described above. On the other hand, these very bright, relatively long-lasting and apparently large balls could represent the upper end of a scale of phenomena in which the 'blob' or 'flow of light' seen by the New Zealand observers, with its low luminosity, lack of well defined shape and location at ground level, could represent the bottom.

While Burbidge and Robertson's observations are of considerable interest, the idea they put forward to explain the motion of the blob — the existence of free magnetic dipoles or permanent current loops in the blob that interact with the magnetic field gradient — would appear farfetched. How could a current loop exist for 15 seconds in a liquid or gas near ambient temperature and pressure? What available liquid or gaseous material could have permanent magnetic dipoles? It is, nevertheless, interesting to put numbers in equation (2) in Burbidge and Robertson's paper. With the horizontal gradients in the vertical field given in Fig. 1 a horizontal force of about 10^{-5} Newtons will be produced by an assumed current of 10 A flowing around the perimeter of the blob. The blob must be slightly more dense than air. If it were less, it would rise. If it were much more, it would appear more like a 'puddle' than a 'blob'. The drag force of the ambient air on such a blob, for the observed velocity of 0.25 m s^{-1}, is about the same as the magnetic force for the assumed 10 A current. Thus, for that value of current, the two forces balance to yield the observed blob velocity. If the vertical gradient in the horizontal field were such as to produce a vertically upward magnetic force of about the same magnitude as the horizontal force, the blob would be levitated if its density were less than 1 per cent greater than air. Otherwise, the magnetic force would be less than the gravitational force, and the blob would remain on the ground. It is worth noting that a 10 A current flowing in an air arc or in an air arc contaminated by vapours of solids produces a level of light output that is painful to look at.

Finally, we should address the question of just what is a ball lightning. There is no consensus! *Nature* is probably the primary publication source for unconventional (if there are truly conventional) ball lightning

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observations and theories. Many of these theories are improbable but do make for interesting reading. A few examples follow. Wooding (Laser Analogue to Ball Lightning, *Nature* **239**, 934; 1972) would have the lightning act as a laser to produce a high-power optical pulse which would ablate a solid surface producing a plasma vortex ring (the ball). The laser radiation would escape at points along the lightning channel when it zigged or zagged. Altschuler, House and Hildner (Is Ball Lightning a Nuclear Phenomenon? *Nature* **228**, 545; 1970) suggest that high-energy protons from lightning collide with air molecules and cause nuclear reactions which produce a high-energy ball. They do note, however, that being near such balls could result in radiation sickness or worse. Ashby and Whitehead (Is Ball Lightning Caused by Antimatter Meteorites? *Nature* **230**, 180; 1971) report the observation of photons at the energy corresponding to position annihilation, and suggest micrometre-size antimatter meteorites as the source for ball lightning. Crawford (Antimatter and Ball Lightning, *Nature* **239**, 395; 1972) has interpreted the measurements of Ashby and Whitehead to suggest that ball lightning is due to the photons produced when positrons in cosmic ray showers (EAS) come to rest and are annihilated.

Ball lightning is a wild and wonderful subject for study. The interested reader can best be introduced to the available theories and experimental data by reading the book by J.D. Barry *Ball Lightning and Bead Lightning* Plenum Press, New York, 1980). ☐

Vive le replicon!

from Ron Laskey and Marcel Mechali

TWENTY years ago Jacob, Brenner and Cuzin[1,2] proposed a fundamental unit of DNA replication, the replicon. They argued that tight coupling of DNA replication to other cellular events was regulated by the action of a diffusible positive factor, 'the initiator', on a specialized chromosomal region, 'the replicator'; resulting in replication of the entire circular chromosome of a bacterium, plasmid or bacteriophage. The twentieth anniversary of the replicon hypothesis was celebrated by a meeting* which considered its impact on current studies of prokaryotic and eukaryotic DNA replication.

The chromosomal replicator of *Escherichia coli* has been mapped to within a 245 base pair (bp) fragment from the *oriC* region. This is sufficient to direct initiation of replication *in vitro*. Surprisingly, polymerization does not begin in this fragment itself but in the upstream flanking DNA, irrespective of the nucleotide sequence of the flanking region (Hirota, National Institute of Genetics, Japan). Replication subsequently becomes bidirectional after an asymmetric start.

The product of gene *dnaA* behaves as an initiator for the chromosomal replicon of *E.coli* and a cell-free system from *E.coli* requires the dnaA protein for initiation at *oriC* (J. Kaguni, Stanford Medical School). Fractionation of this system has produced an intriguing result. As well as several identified components[3] and at least two unidentified positive factors, an additional template-specificity factor is required for initiation at the *E.coli* replication origin. In the absence of this factor the amount of DNA synthesis increases, but synthesis no longer requires the *dnaA* gene product and it is no longer template specific for *oriC*. Apparently the enzymes of replication could initiate elsewhere and the replicon concept has acquired another component which constrains initiation to the site specified by the initiator.

In the initiation of DNA replication two discrete roles for RNA synthesis have been identified. First, DNA chains are usually primed by synthesis of oligoribonucleotide primers[4]. Second, there are several examples of transcriptional activation of DNA replication in which the origin region is transcribed to form a transcript which allows a true priming event subsequently to occur. A particularly good example is phage λ where a pause during transcription through the origin appears to expose the site for priming in a single-stranded form (Hobom, Albert Ludwigs University, Freiburg). Alternatively, in the replication of plasmid Col E1, interaction of the long RNA primer with a complementary transcript from the opposite strand controls plasmid copy number (Tomizawa, NIH). In phage T7, two promoters for T7 RNA polymerase flank the primary replication origin and appear to be involved in replication initiation. They have no open reading frame between them and their deletion inhibits replication from that origin (Richardson, Harvard Medical School). Conversely, deletion of the primary replication origin results in initiation at alternative sites which are also near promoters for T7 RNA polymerase. During elongation nascent T7 DNA chains are primed by the T7 gene 4 protein which could use its associated helicase activity to unwind DNA, thus exposing primase sites (Richardson). In T4 the virus-coded primase and helicase are different proteins which interact during replication (Alberts, UCSF Medical School).

Studies of replication in eukaryotes have lagged far behind, but promising experimental systems are now emerging for studying several aspects of the process including RNA priming. Thus Conaway (Stanford Medical School) reported that the primase activity from *Drosophila* co-purifies with DNA polymerase α, the polymerase primarily responsible for replication in eukaryotes.

A clear exception to the rule of RNA priming is provided by adenovirus, and a controversy has existed over whether the DNA polymerase which replicates adenovirus DNA is α or γ. This appears to have been resolved by the finding that it is a separate polymerase encoded by the virus itself (Hurwitz, Albert Einstein College, New York). The polymerase is complexed with the 'pre-terminal protein' which binds dCTP and then attaches covalently to the ends of the linear genome during priming. In the presence of the virus-encoded DNA-binding protein and two other factors, full-length copies of the adeno genome can be synthesized *in vitro* from purified components.

Recent studies in yeast may advance our knowledge of eukaryotic replicons. Transformation assays have identified a limited subset of DNA segments which allow plasmids to replicate autonomously in yeast. These autonomously replicating sequences have the properties expected of chromosomal 'replicators'. Therefore evidence that replication initiates at these sequences is important and Campbell (CalTech) described a cell-free system which appears to initiate preferentially at autonomously replicating sequences *in vitro*. An additional advantage of yeast is the advanced state of yeast genetics and a permeabilized cell system derived from a thermosensitive cell-cycle mutant (cdc8) which can be complemented by fractionated extracts from wild type (Campbell). The transformation assay also led to the isolation of yeast centromere sequences which confer mitotic and meiotic stability on plasmids replicating in yeast. Carbon (University of California, Santa Barbara) reported another effect of the centromere — control of copy number of the endogenous 2μ plasmid of yeast. When a centromere is introduced, the copy number falls from around 50 to only 1 per cell.

Yeast has advantages over higher eukaryotes for the study of replication but Hamlin (University of Virginia Medical School) described a promising approach for studying a replicon in Chinese hamster ovary cells. The gene coding for dihydrofolate reductase becomes amplified in response to methotrexate selection. The degree of amplification

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* A meeting entitled 'The Replicon, 20 years after' was sponsored by INSERM at Seillac, France, on 19–23 September 1982.

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allows restriction fragments from this gene and its flanking sequences to be recognized in digests of total DNA. Pulse-labelling with thymidine reveals that the characteristic restriction fragments replicate early in the S phase of the cell cycle and that there is a temporal order of fragment labelling. Isolation of these fragments and insertion into other cells could help in elucidating the nature of the elusive eukaryotic 'replicon'.

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Is polypeptide heterogeneity the key to neurofilament function?

from Rosaleen Calvert

THE most enigmatic component of the cytoskeleton remains the intermediate filament. Microtubules and microfilaments can depolymerize to tubulin and to actin respectively, and the effects of their absence in the cell investigated. In contrast, there are no situations where intermediate filaments depolymerize *in vivo* (though other ultrastructural changes do occur) and no known drugs which have this effect. Nonetheless, by using a variety of approaches we are now beginning to find out what the functions of these filaments may be. One class of intermediate filaments — the neurofilaments — is the subject of this article (see also refs 1,2).

Intermediate filaments were first defined ultrastructurally as filaments intermediate in width between the thick filaments of myosin and thin filaments of actin[3]. Subsequent purification from different tissues led to the surprising observation that although the proteins actin and tubulin always consisted of one and two polypeptides respectively, there were five classes of intermediate filaments. In the case of the epithelial cytokeratins, they contain at least nineteen different polypeptides in contrast to, for instance, the fibroblast intermediate filament protein vimentin which is a single gene product. The three remaining intermediate filaments are desmin in muscle, glial fibrillary acidic protein in glia and neurofilaments. Mammalian neurofilaments consist of only three polypeptides of molecular weight about 200,000, 150,000 and 70,000 (ref.4); these will subsequently be referred to as the 200K, 150K and 70K. It has proved fruitful to probe their different roles in neuronal structure and behaviour.

The first indication of heterogeneity in neurofilament structure came from a genetic polymorphism of the 200K in rabbits, in which one variety had a molecular weight about 10,000 larger than the other. Heterozygotes and homozygotes for each allele were observed. Unfortunately, no functional attributes of these animals mirror their molecular differences[5]. There is further heterogeneity in the isoelectric points of all three polypeptides[6], but again, the functional significance is not yet clear.

Recently, immunofluorescence has revealed neuronal processes which lack or have very little of the highest-molecular-weight polypeptide — the dendrites of pyramidal cells of the cerebral cortex and hippocampus and certain cell bodies or axons in the brain stem on sections of adult rat[7] and human[8] brain, and certain dorsal root ganglion[9,10] neurones. In developing rat brain the 200K can be first detected with Coomassie brilliant blue stain on a gel three days postnatum, whereas the 150K and 70K can be seen one day prenatum. It could be therefore that the 200K is found only in mature axons[11]. However neurofilaments are known to be susceptible to an endogenous calcium protease and there is some evidence that this is not evenly distributed, at least within single neurones[12], so it is possible that the 200K is proteolysed during tissue preparation.

Ten nm filaments can be reconstituted from the 70K alone[13-15] or with the 200K added, and thin sections show projecting side arms[13], suggesting that the 200K could be responsible for the bridges between neurofilaments observed in the electron microscope. There is some immunological evidence for this: antibodies specific for the 200K appear to label the cross-links between neurofilaments in electron microscope studies of whole neuronal cytoskeletons[16]. Moreover, *in vitro* digestion studies using trypsin have shown that 75 per cent of both the 200K and the 150K can be removed, leaving morphologically intact filaments[17].

It is interesting that in the growing axon, the neurofilament triplet is transported distally at eight times the final rate. Retardation correlates with the increasing quantities of the 200K which appear at this time, and it has been proposed that a concomitant formation of cross-bridges could be the explanation for the change in rate of axoplasmic transport[18]. This anterograde transport of neurofilament polypeptides also raises the question of what happens to the filaments or networks of filaments when they reach the axon terminal. Soluble subunits have never been found, so degradation is still considered as the most plausible mechanism for their disposal[19]. In support of this mechanism is the observation that a monoclonal antibody[20,21], which binds strongly to the 200K and weakly to the 150K, binds to these and only two other polypeptides in neonatal rat brain whereas in the course of further development a whole ladder of antibody-binding polypeptides appears below the 200K on polyacrylamide gels. A possible interpretation of this observation is that gradual cessation of axonal outgrowth necessitates an increase in degradative processes to dispose of neurofilaments accumulating at the axonal terminals. The 200K polypeptide thus emerges with a distinct structural role. Further compari-

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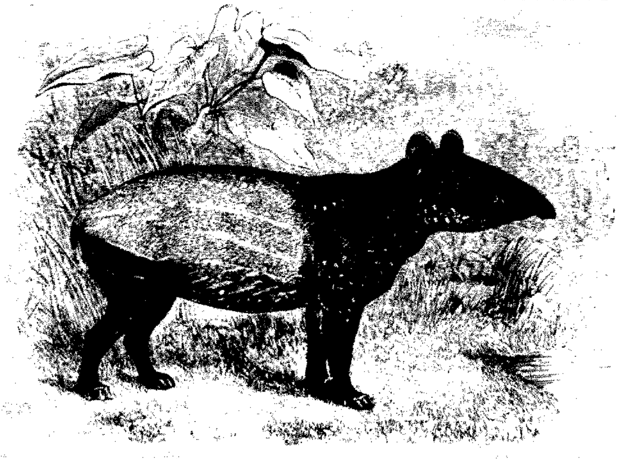
100 years ago

NEW OR RARE ANIMALS IN THE ZOOLOGICAL SOCIETY



THE MALAYAN TAPIR (*Tapirus indicus*). There is no better instance of discontinuous distribution on the world's surface than that of the Tapirs. While Tropical America contains several species of *Tapirus*, and may be regarded as the *focus* of the genus, a single well-marked species occurs in Tropical Asia. This is the Malayan or Indian Tapir, *Tapirus indicus* (sive *malayanus*) of systematists. From *Nature* **27**, 151, 14 December 1882.

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son of the situations in which it is present or absent may provide more insight into its function.

Special features of the other two neurofilament polypeptides are scarce. In a study of polypeptide compositions of different sections of the axons in a mouse optic tract, the 150K exhibited microheterogeneity. Two major species were visible along the length of the optic pathway with an extra one appearing distally. The amount of this third polypeptide could be increased by incubation of the intact optic pathway *in vitro* with a calcium-containing buffer, suggesting the presence of a protease which was absent or inactive in the proximal part of the axons[12]. This again supports the idea that neurofilaments are degraded as they reach the axon terminal.

The anti-neurofilament monoclonal antibody[20] mentioned above also binds to a high-molecular-weight polypeptide present in developing rat brain and absent in other tissues of neonatal rat. Thus by immunological criteria there seems to be another neurofilament polypeptide associated with immature brain[21]. Whether other properties of this polypeptide, such as solubility and secondary structure, will confirm its assignment as an intermediate filament protein remains to be seen.

An interesting selection of rather different neurofilament structures is now accumulating. Those situations where a change in structure can be seen and correlated with a physiological event offer the best chances of using structural differences to throw light on the elusive neurofilament function. □

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Collagen genes

from Paul Tolstoshev and Ellen Solomon

THE collagen genes, a major family of at least fifteen genes found throughout the vertebrate and invertebrate kingdoms, have been much studied, not only for what they can tell us about the molecular biology of evolution but also because they are developmentally regulated, crucial for the structural integrity of most tissues and are implicated in many human mendelian genetic disorders. Reports delivered at a recent meeting* revealed progress in understanding of all these areas.

All collagenous proteins have in common a triple-helical region with a repeating pattern in which every third amino acid is glycine. The interstitial collagens (types I, II and III) form rigid rod-like structures while basement membrane collagen (type IV) forms a different kind of molecule, still with large amounts of triple helix, but with nonhelical interruptions.

A very detailed picture of the gene for the α_2 chain of type I collagen[1-3] is now available (H. Boedtker, Harvard University). The most remarkable feature of this gene, apart from its size and that it contains about 50 introns in 40 kilobases (kb) of DNA, is the unique conservation of the 54 base pair (bp) exon size throughout the α-chain-coding region. There has been much speculation as to the significance of this structure in the evolution of collagen genes[4,5] and the analysis of other collagen genes has been awaited with great interest. Information is now available for mouse α_1 (I) (ref. 6), chick α_1 (II) (B. Upholt, University of Chicago), chick α_1 (III) (Y. Yamada, NCI, Bethesda), human α_2 (I) (F. Ramirez, Rutgers University) and an α_1 (I)-like gene[7]. All these genes have conserved the basic 54 bp (18 amino acid) exon size in the triple-helical coding region of the gene. The size of this exon and its variations [45 bp (54 − 9), 108 (54 × 2) and 99 (108 − 9)] are similar to those found in the chick α_2 (I) collagen gene. The only permissible deletions thus appear to be of size 9 bp — one Gly-X-Y triplet. Comparison of particular exons among the different genes indicates that sizes and position are also strongly conserved. Intron sizes, however, vary enormously, from less than 100 bp to several thousand. In general the data are compatible with the idea that the genes had their origin in an ancestral 54 bp sequence which expanded by repeated duplications, but was constrained by the absolute necessity of maintaining the repeating Gly-X-Y structure in the protein.

Another remarkable feature of the evolution of the collagen genes is the way that the amino acid sequence and nucleotide sequence have been conserved around the carbohydrate attachment site in the C-terminal propeptide region in chick α_2 (I) and human α_2 (I) (D. Prockop; Rutgers University). Similar conservation in this region has been observed in the sheep α_2 (I) gene[7], the chick α_1 (II) gene (B. Olsen, Rutgers University) and the chick α_1 (III) gene. It is easy to think of functional reasons why such conservation occurs at the amino acid level but lack of third-position codon change in this region is striking, implying selection at the nucleotide level for the conservation of the sequence and making the region a fascinating one to explore — it may perhaps function as the attachment site for a regulatory molecule.

Do all collagen genes have the same basic structural features and have a common evolutionary history? From recent studies on the structures of fruitfly[9,10] and nematode[11] collagen genes, the answer to at least the first question is clearly no. The cuticle nematode *C. elegans* contains a large number of collagenase-sensitive cuticle proteins, and the genes for two of these have been isolated (D. Hirsch, University of Colorado). These two genes are not closely linked, are related but not identical and are each about 1,200 bp long. Sequences coding for Gly-X-Y repeats are seen in both of the genes, but none of the exons is 54 bp or variants thereof, or a multiple of 9 bp. In fact, the first gene is divided into only three exons, the second into two. Analysis of a fragment of a *Drosophila* collagen gene (J. Monson, University of California, San Francisco) has revealed two large regions, of 662 and 726 bp, which encode a region of 460 amino acids. The structure of this gene is again quite different from that of the vertebrate interstitial collagen genes. The *Drosophila* collagen protein appears to be closely related to basement membrane type IV collagen (J. Fessler, University of California, Los Angeles) and cross-reacts with antibodies to mouse type IV collagen. The big question now is: what is the structure of the genes for vertebrate basement membrane collagens — are they similar to the interstitial collagen genes or like that of the nematode and *Drosophila?* The answer obviously is crucial to our understanding of the evolution of collagen genes.

Analyses using dot hybridization on genomic human placental DNA (R. Dalgleish, NHLBI, Bethsda) indicate that

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there is only one copy of each of the α_1 (I), α_2 (I) and α_1 (I)-like genes per haploid genome in humans. The genes coding for the two chains of human type I collagen are located on different human chromosomes[12,13] α_2 (I) on chromosome 7 and α_1 (I) on chromosome 17 (E. Solomon, ICRF, London; F. Ramirez). The presence of the genes for the different chains of heteropolymer on different chromosomes has many well known precedents, and does not allow prediction of whether other related genes will be clustered near either gene.

One of the most studied systems of collagen gene regulation has been the Rous sarcoma virus (RSV)-transformed chick embryo fibroblast (CEF) where the production of type I collagen is dramatically decreased on transformation, probably by regulation at the transcriptional level[14,15]. The availability of temperature-sensitive strains of the virus has allowed analysis of both the induction and reversal of the transformed state (M. Sobel, NIDR, Bethesda) and has prompted studies of the transcriptional regulatory signals of the chick α_2 (I) collagen gene. The promoter of this gene has been inserted into a hybrid SV40/pBR322 shuttle vector (B. de Crombrugghe, NCI, Bethesda) which makes possible detection of promoter activity by measurement of the activity of the product of the *Escherichia coli* chloramphenicol acetyl transferase gene. Analysis of deletion mutants in the α_2 (I) collagen promoter region of this vector has provided some evidence for regulatory sequences, particularly those potentially capable of forming hairpin structures[16]. Neither this approach, nor attempts to correlate methylation of the α_2 collagen gene with its activity[17] (C. McKeon, NCI, Bethesda), have been successful in yielding clues to the precise nature of transcriptional regulatory mechanisms. However, a role for methylation in the reduction of activity of type I procollagen gene expression on transformation of human lung fibroblasts with SV40 has recently been reported[18].

Chick embryo chondroblasts, which normally produce type II collagen, when transformed with RSV strain show decreased synthesis of type II collagen and decreased levels of type II collagen mRNA (ref. 19 and S. Adams, University of Philadelphia). Surprisingly, type I collagen mRNA begins to accumulate, but is not used by the cells to make type I collagen. This is clearly a regulation at the level of translation. Other examples of translational regulation are seen in the effects of the N-terminal propeptides on collagen synthesis in normal and dermatosporatic sheep fibroblasts (P. Müller, Max Plank Institut, Munich) and in the regulation of collagen gene expression in fetal sheep skin development[20] and human lung fibroblasts[21]. Clearly, continued analysis of these and other systems using cloned probes will reveal the exact details of the variety of mechanisms cells use to modulate their collagen production.

A variety of human genetic disorders may be due to a structural defect of collagen. These include Ostrogenesis imperfecta, several of the Ehlers-Danlos syndromes and the Marfans syndrome. These disorders, while normally inherited in simple mendelian fashion, vary greatly in the severity of their clinical symptons. Biosynthetic studies of collagen production in cultured fibroblasts from affected individuals show abnormal secretion of collagen in all the three types of disease (P. Byers, University of Washington). The abnormality may be due to underproduction or lack of one constituent α chain, an abnormally long or short chain causing incorrect assembly of the precursor, or inefficient cleavage of the terminal peptides. The wide variability of symptoms has long caused difficulties of classifications of heritable disorders of connective tissue and of meaningful genetic counselling. Classification using biochemical methods is being attempted (D. Rowe, University of Connecticut), but DNA analysis is obviously of prime importance in the understanding of these disorders. Such analyses for two individual cases were reported. A fibroblast culture from an affected individual with the severe perinatal lethal form of OI type II demonstrated both shorter mRNA molecules for α_1 (I) chains and a deletion in the α_1 (I) chain gene in the DNA (M.L. Chu, Rutgers University). The second case is another, less severe form of Ostrogenesis imperfecta, where, remarkably, no α_2 (I) collagen chains are found at all in the skin[22]. While this case is obviously also a prime suspect for a deletion, in fact both pro-α_1 (I) and pro-α_2 (I) mRNAs are present in normal amounts in fibroblasts (D. Prockop, Rutgers University). An intriguing study was mentioned (P. Byers; J. Phillips, John Hopkins University) of a large Osteogenesis imperfecta family in which the disease segregates completely with a restriction site polymorphism near a growth hormone gene on chromosome 17. The suggestion is one of linkage of the disease gene (possibly the α_1 (I) chain gene) with the polymorphism, and is the first instance of this kind of analysis for a connective tissue disease. The analysis of more cases of the genetic connective tissue disorders is underway with the probes that are available, and the results will be of great basic as well as clinical interest. □

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Onco Gen

from Peter Newmark

A paper on page 607 of this issue implicates the cellular gene known as c-*mos* in malignancies of antibody-producing cells or their precursors and illustrates a novel way of activation of an oncogene.

The c-*mos* gene is so called because it is the cellular homologue (and presumably the precursor) of the oncogene carried by the Moloney murine sarcoma virus. Whereas the viral gene is oncogenic, c-*mos* is not unless activated. In 1980 Blair *et al.* (*Science* **212**, 941) demonstrated activation of c-*mos* by the attachment to it of the long terminal repeat sequences of the Moloney murine sarcoma virus, probably on account of increased expression of c-*mos* under the influence of the long terminal repeats.

Rechavi *et al.* now demonstrate a completely different mechanism of activation of c-*mos* by — strange though it may seem — the replacement of a sizeable chunk of the gene by extraneous DNA. The mechanism is all the more important because it is not the result of an artificial construct but has been discovered in a chemically induced mouse myeloma.

The evidence comes from a comparison of the sequences of the normal c-*mos* gene and an abnormal version in the myeloma. The abnormal version, which was able to transform NIH 3T3 fibroblasts — the standard assay for an oncogene — had lost over 500 nucleotides from one end, including the information for the first 88 amino acids of the c-*mos* protein.

Intriguingly, a 159-nucleotide block of DNA immediately adjacent to what is left of c-*mos* has the hallmarks of a transposable genetic element, suggesting that a transposition has displaced some c-*mos* nucleotides and activated what is left.

The mechanism of the presumed activation is as much, if not more, of a mystery than in the case of the point mutation that seems to have produced the human bladder carcinoma oncogene (*Nature* **300**, 143 & 149).

In a note added in proof, Rechavi *et al.* claim that they have also detected a rearranged c-*mos* gene in a Burkitt's lymphoma. It is not yet known if the rearrangement involves a transposition but it does seem to be the consequence of a translocation from chromosome 8 to 14, bringing the c-*mos* gene into the proximity of the variable-region heavy-chain immunoglobulin gene in a fashion similar to that already reported for the c-*myc* gene (see *Nature* **300**, 403). □

Peter Newmark is deputy editor of Nature.

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REVIEW ARTICLE

The T lymphocyte antigen receptor—paradigm lost

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It has been dogma that the antigen receptors on T lymphocytes must have recognition sites that are essentially the same as those of antibodies and that the receptors would be found if structures related to immunoglobulin V-domains could be identified on T lymphocytes. In a critical assessment we conclude that there is no sound basis for these views.

ANTIBODIES are the only well-characterized molecules that are known to bind foreign antigens in the immune response. An enormous range of antibodies can be produced with their different specificities being due to different amino acids in the combining site.

Lymphocytes are the only cells known to be capable of specific recognition of foreign antigen. This recognition results in the stimulation of antibody secretion and the activation of cells responsible for cell-mediated immunity. B lymphocytes (bursal or bone marrow-derived) give rise to antibody-secreting cells while T lymphocytes (thymus-derived) are responsible for the cell-mediated functions which include cytotoxicity and the control of other immune responses. Helper T cells provide a stimulus which is usually essential for the differentiation of B cells and cytotoxic T cells. Suppressor T cells turn off immune responses.

The antigen receptor on B lymphocytes is antibody and it has long been argued that the antigen receptor of T lymphocytes should at least use the antibody gene pool to encode its combining site regions. This point of view derives from the argument that it is unlikely that the problem of recognizing antigen should have been solved twice in evolution. However, we conclude here that there is no good evidence for the expression of antibody genes in T cells and many data suggest the contrary. Furthermore the discovery of molecules related to immunoglobulin (Ig) does not necessarily lead to the identification of immune receptor molecules.

Receptors on B lymphocytes

Figure 1*a* shows a model for a receptor IgM at a cell surface[1,2] and defines the terms used to describe antibody structure. The

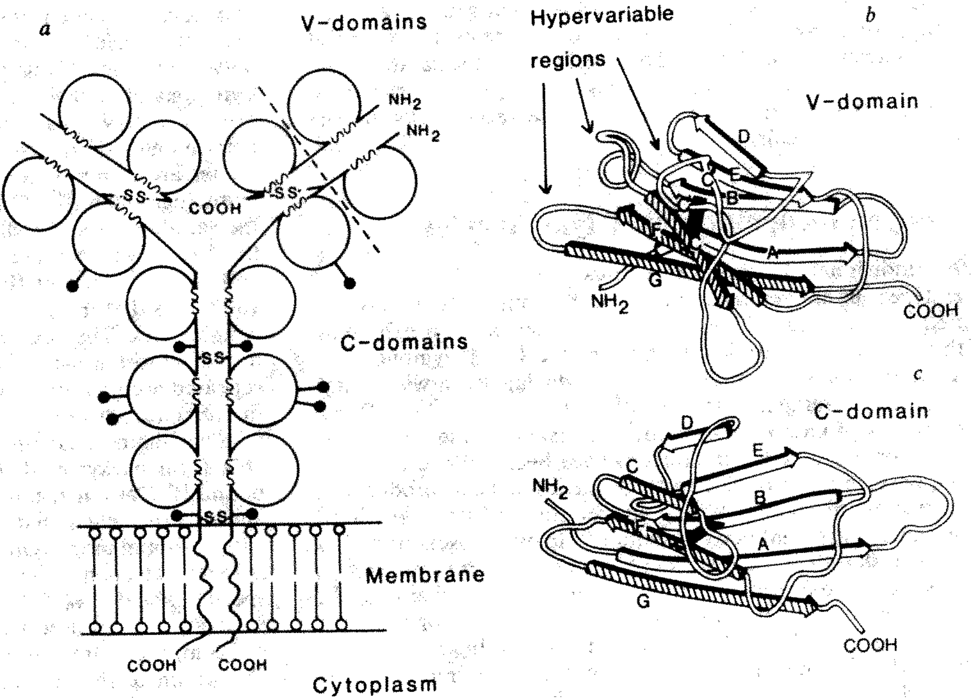

Fig. 1 The structure of receptor antibody. *a* Shows IgM antibody at a cell surface. The structure consists of two identical heavy (H) and two identical light (L) chains held together by disulphide bonds (SS symbols between the chains). There are two identical antigen combining sites, each formed from the N-terminal parts of one H and one L chain. The H and L chains can be divided into domains which show sequence homology (illustrated by circles) including a conserved intra-chain disulphide bond (≀ symbols). The N-terminal domains are called V domains (or regions) because their amino acid sequence varies to give different antibody combining specificities. The other domains are called C domains because their sequence is constant for any one L-chain type or H-chain class. The C domains for any one chain are collectively referred to as the C region. The symbols ⫯ show attachment points for N-linked carbohydrate structures. *b* And *c* show the folding pattern for the polypeptide of V and C domains respectively. In both sorts of domain anti-parallel β-strands form two β-sheets that are held together by hydrophobic interactions and the conserved disulphide bond. The bar indicates the conserved intra-chain disulphide bond and the letters, the β-strand sequences labelled sequentially along the sequence. The V domain has an extra loop of sequence in the middle and the β-strands in this are indicated by C′ and C″. The loops of sequence in V domains which are believed to form the combining site show great variation in amino acid sequence between different V domains and are indicated with the label: hypervariable regions (the domains are redrawn from ref. 3).

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Table 1 Chromosomal assignments of genes for immunoglobulin and immunoglobulin related molecules in the mouse

Molecules		Chromosome (ref.)
Immunoglobulins		
Lambda:	$V_\lambda C_\lambda$	16 (152)
Kappa:	$V_\kappa C_\kappa$	6 (153)
H-chains:	$V_H C\mu\delta\gamma_3\gamma_1\gamma_{2b}\gamma_{2a}\varepsilon\alpha$	12 (154)
Major histocompatibility complex antigens		
Class I:	H chains	17 (19)
Qa:	H chain	17 (155)
Class II:	α and β chains	17 (19)
β_2-microglobulin		2 (156)
Differentiation antigen		
Thy-1		9 (19)
T receptors		?

antibody heavy (H) and light (L) chains can be subdivided into domains of about 110 amino acids which show sequence and structural homology[3,4] (Fig. 1*b*, *c*) but have quite different functions. The variable (V)-domains form the antigen recognition site while the constant (C)-domains of the H-chains function as determinants to be recognized by molecules of the immune effector systems (for example the complement cascade) which are triggered after antibody has bound antigen[5,6].

The genes for the V and C regions of any one immunoglobulin chain are linked but separated by a considerable distance on the chromosome. There are multiple *V*-domain genes but only one *C*-region gene for each chain. In ontogeny of B lymphocytes one V_L and one V_H gene is spliced to a new position closer to their respective *C*-region genes, committing the cell to one combining site[7,8]. In further differentiation genetic translocations can lead to expression of different C regions in the H chain[9] but the expressed V_H and L chains remain the same unless somatic mutation occurs.

The commitment of B cells to one combining site specificity can be demonstrated at the cellular level. Antibody at the B-cell surface can bind soluble or particulate antigen[10,11] and the antigen-binding cells for a typical antigen are usually found at a frequency of about 10^{-4}. These cells can be separated from other lymphocytes in various ways and have been shown to include the precursors for antibody-secreting cells of the appropriate specificity[12–14].

Antigen recognition by T lymphocytes

An individual T lymphocyte must have a limited set of endogenous antigen receptors since the immune functions carried out by T-cell clones are antigen specific[15]. In exhibiting this clonal specificity T lymphocytes are like B lymphocytes; another similarity is that T cells can distinguish a wide range of different antigenic structures[16–18]. However, T- and B-cell responses often do not coincide. For example a large number of minor transplantation antigens have been defined by T-cell responses but antibodies to them have not been produced[19]. Also some histocompatibility antigen mutants provoke T-cell responses but no antibody formation[20]. In other cases antibodies distinguish different antigens but T cells show cross-reaction[16]. This is commonly seen in immune responses to viral antigens[21].

One major difference between B cells and helper or cytotoxic T cells is that these T cells cannot directly bind soluble or particulate antigen that has not been processed by macrophages. Past claims to have identified antigen-binding T lymphocytes have been, at best, controversial. Acquired antibody can easily lead to false positives[22,23] as can the fact that the commonly used antigen TIGAL may bind to small numbers of T lymphocytes by cross-reaction with Fc receptors[24]. Acquired antibody is not a problem in studies with bursectomized chickens which have functional T lymphocytes but no B lymphocyte serum antibody. Antigen-binding T cells cannot be detected in these animals[22,24–26]. Similarly in mice there is no evidence for direct binding of antigen by helper or cytotoxic T cells if the criterion used is the enrichment or depletion of functionally active T cells on the basis of antigen binding[17,27–31].

The situation is different for suppressor T lymphocytes, which do show antigen binding[28,32,33] and also secrete factors which bind antigen[34]. In these properties suppressor T cells are similar to B lymphocytes and this analogy is supported by the finding that suppressor cells distinguish native and denatured antigen (as do B cells) whilst helper T cells do not make this distinction[28].

The difficulty in finding antigen-binding T cells became explicable when it was shown that T lymphocyte helper and cytotoxic responses require simultaneous recognition of foreign antigen along with self-histocompatibility antigen at the surface of an antigen-presenting cell[35,36]. Functional T lymphocytes will bind to a monolayer of macrophages which have been loaded with antigen[37]. Cytotoxic T lymphocytes usually recognize antigen along with class I histocompatibility antigens whilst recognition by helper T lymphocytes usually involves co-recognition of class II antigens[36].

The differences in antigen recognition by T and B cells suggest major differences in their receptors but do not rule out the possibility that T-cell receptors utilize at least a part of the antibody structure. Initially it seemed possible that T receptors were very similar to antibodies but that they possessed a different H chain. This is the IgX concept[38] (Fig. 2*a*) and is based on the fact that the different classes of antibodies are specialized for different effector functions. Subsequently the IgX model was modified to that shown in Fig. 2*b* in which the only immunoglobulin products shared with B cells are the V_H domains[39–41].

The search for T lymphocyte immunoglobulin

Antibodies have been widely used to search for immunoglobulin on T cells. Antibodies combine with a relatively small area of an antigen and antibodies against immunoglobulin are likely to recognize sequences at the exposed corners or surfaces of the immunoglobulin domains (Fig. 1*b*, *c*). By immunization between species it is simple to produce antibodies reacting with C regions of L chains and H chains and it is also possible to prepare anti-V-region antibodies which react with conserved determinants shared by many antibodies. In fact, anti-V antibodies are common components in sera raised against whole immunoglobulin[42,43]. Other anti-V antibodies can be against the variable parts of V domains (anti-idiotypes, see below) and these react with a minor sub-set of total immunoglobulin[44,45].

If the IgX concept (Fig. 2*a*) is right, anti-immunoglobulin antibodies that include anti-L-chain antibodies should label T lymphocytes. The failure to detect such labelling[25,46], by any but the most sensitive methods, must be one of the most repeated observations in immunology. In experiments using the fluorescence activated cell sorter the labelling of T lymphocytes with anti-immunoglobulin antibody is invariably indistinguishable from background (for example, ref. 47). If the T cells bound 10% as much antibody as B cells, this would be detected and it is thus clear that T lymphocytes do not routinely label with anti-immunoglobulin reagents at 30% of the B lymphocyte level as has been claimed[48]. However, using autoradiography specific labelling of T lymphocytes with anti-immunoglobulin antibody can be detected at about 100–1,000 molecules per cell compared with about 10^5 molecules for B lymphocytes[49,50]. The origin of the immunoglobulin detected has been studied in the rat using anti-L-chain allotype antibodies[51] and in the chicken using normal and bursectomized birds[52]. In both cases it was concluded that the small amount of immunoglobulin detected was acquired from exogenous sources. L chain was also searched for in extracts from T lymphocytes of the bursectomized chicken and <100 molecules per cell was detected[53].

To our knowledge there are no experiments in which the serological determinants of any immunoglobulin constant region have been found on T lymphocytes and been shown to be of endogenous origin (see controversial positives, below).

Given the above results and data from experiments with anti-idiotype antibodies[39,40] (see below), a model for the T-cell receptor as shown in Fig. 2*b* became accepted[41]. This is characterized by the expression of antibody V_H regions which should be detectable with anti-V-region antibodies specific for conserved determinants. Suitable antibodies can be prepared against the rabbit 'a' allotypes which are polymorphic determinants common to all immunoglobulin classes[54] and expressed on isolated H chain and V_H fragments[55]. Using these antibodies, attempts have been made to find molecules with V_H but not L-chain determinants by labelling of rabbit lymphocytes and analysis of cell extracts. In seven independent studies (refs 56–62) no evidence was found for these molecules, although expression at a few thousand molecules per cell may have gone undetected. Stimulation *in vivo* with antigen[62], or *in vitro* with mitogens or allogeneic cells[63] did not alter this negative result.

One report describes antigen-binding rabbit lymphocytes which express V_H but not L-chain determinants[64] and another, antigen binding molecules with these characteristics[65]. These results are difficult to evaluate without further analysis and an understanding of the nature of antigen binding T cells and factors (see above).

Other antibodies which react with V domains of a large fraction of immunoglobulin have also been produced. Two groups have used chicken or mammalian antibodies against rabbit V_H determinants distinct from the 'a' allotypes including antibodies that reacted only with free V_H or H chain[60,63]. V_H unassociated with classical immunoglobulin was not detected. Others have prepared antibodies against mouse V domains. A monoclonal anti-mouse V_H antibody did not react with T cells[66] and rabbit anti-mouse F_V (includes V_H and V_L) did not label thymocytes or T cells[67]. In contrast, similar rabbit antibodies against mouse V domains have been reported to inhibit antigen binding by T cells[68], but to show no binding to T-cell clones[17]. Again the positive effect is difficult to interpret due to the uncertainties about antigen-binding T cells. A rabbit anti-human V_H antibody was reported to show inconsistent labelling of human T cells[69].

To summarize, the evidence for V domains on T cells from experiments with widely reacting anti-V antibodies is unconvincing (particularly since there are a variety of ways that false positives can occur, see below).

Experiments with nucleic acid probes

Nucleic acid probes provide alternative reagents for searching for the expression of antibody genes in T cells. The probes can be used to search for mRNA in the T-cell cytoplasm or to examine the DNA of T cells to see if V-region genes have been moved in the manner that occurs in the commitment of B lymphocytes. In preparing probes DNA is copied (cDNA) from purified immunoglobulin mRNA and then cloned in plasmids to ensure the purity of the probe. Problems with the purity of T cells remain although B-cell contamination is excluded if T-cell clones or lines are studied.

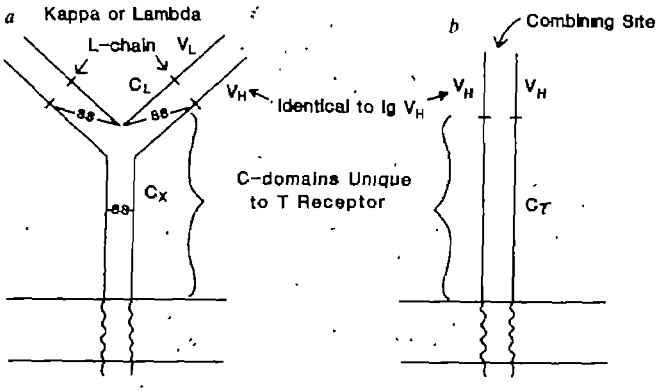

Fig. 2 Models for immunoglobulin related T lymphocyte antigen receptors. In both models the polypeptides differ from the homologous regions of B-cell antibody only in in C_χ or C_τ regions which are the products of postulated genes for constant regions expressed only in T lymphocytes (*a* based on ref. 38, *b* on refs 39–41).

Using cloned cDNA probes L-chain mRNA has not been detected in any T-cell lines[70,71]. Small amounts of κ mRNA were found in thymus[71–73] cell extracts but are likely to have come from plasma cells[72]. In a study using uncloned κ cDNA a reaction was detected in most thymus cells by *in situ* hybridization[74]. This result could be due to minor contaminants in the probe. RNA for the C region ($C\mu$) of IgM H chain has been detected in a proportion of T-cell lines[70,75] and thymocytes[72,73]. This $C\mu$ RNA is not likely to be B-cell-derived[72]. However, similar mRNA was also found in some myeloid cell lines[70] and in the T-cell lines no evidence for synthesis of $C\mu$ polypeptide was found in a rigorous study[76]. The mRNA detected did not appear to contain V_H sequences[77] and it seems to be an aberrant product made in a variety of early haematopoietic cells. V_H mRNA was not detected in cloned T cells or T hybridomas or functional T-cell lines in a study where four cloned V_H probes were used in conditions that allowed detection of a high proportion of all V_H mRNA in B cells[78].

Analysis of T-cell DNA showed no rearrangement of L-chain genes as occurs in B cells before immunoglobulin expression[71,79,80]. Also some studies found no rearrangement of H-chain genes[71,80,81]. In other cases, H-chain genes of some, but not all, clones or cell lines showed some rearrangement[75,79,82,83]. In B cells the construction of a functional H-chain gene involves splicing of a V_H segment, which codes for most of the V-region sequence, to a diversity (D) segment and a joining (J) segment[8]. In T-cell clones which showed V_H rearrangement splicing of the D and J segments occurred but rearrangement to produce a functional V_H gene was ruled out in detailed studies on two cytolytic T-cell lines[83].

At present, therefore, nucleic acid probes have not clearly aided the problem of identifying the T-cell receptor.

Controversial results not involving idiotypic determinants

The above data indicate that antibody gene products are absent from T cells. Yet, a number of reports claiming identification of T cell immunoglobulin or of cross-reacting molecules of relevance to the T receptor have to be considered.

Anti-immunoglobulin antibodies raised between species widely separated in phylogeny can give heavy labelling of thymocytes or T lymphocytes[84–87]. However, the reaction of rabbit antibodies against frog[88,89] or trout[90] immunoglobulin with thymocytes was shown to be due to a sub-fraction of antibody reacting with carbohydrate. A mouse monoclonal antibody against frog IgM did not label frog thymocytes[91]. The labelling of mouse thymocytes with chicken anti-mouse immunoglobulin antibodies was also due to anti-carbohydrate antibody[92]. This was of particular interest since the antibodies analysed were amongst those used by Szenberg *et al.* in their studies[87] claiming detection of mouse T-cell immunoglobulin.

Other claims for positive results have been made when immunoglobulin was detected by immunoprecipitation in extracts from T lymphocytes or thymocytes. Cone and Marchalonis have consistently reported positive results in studies[93–95] using cells that have been surface-labelled with ^{125}I. A number of other groups have not supported these findings[96–98]. Cone attributed success to the methods used to extract membrane proteins. Urea-acetic acid was initially favoured[93] and subsequently very low levels of detergent were recommended[94]. However, quantitative studies showed urea–

acetic acid gave very poor recovery of B-cell immunoglobulin[53,99,100] and very low levels of detergent might release membrane proteins as aggregates[101]. This could give misleading immunoprecipitations.

In biosynthetic labelling studies immunoglobulin has been detected in extracts from thymus cells[102,103]. However, the amount of thymus-derived immunoglobulin varies greatly between animals[53,100] and cannot be detected if thymus cells are treated to remove B cells before preparing extracts[98,104]. Relatively large numbers of plasma cells can be found in the thymus[105] and these are likely to be the source of thymus immunoglobulin. Removing all contaminating B cells is difficult since not all plasma cell precursors are readily removed by procedures commonly used to deplete Ig^+ cells from a lymphocyte mixture[106].

Finally, a general problem is the possibility of cross-reactions with the many immunoglobulin related membrane molecules (Table 1) which are not relevant to the T receptor. Anti-κ chain antibodies which react with β_2-microglobulin have been described[107,108].

Idiotypic determinants on T lymphocytes

Idiotypic antigenic determinants are displayed in the V regions of antibodies and may often be located in the loops of amino acid sequence making up the combining site[44,45]. In many—although not all[109,110]—cases they correlate with the combining site specificity of the idiotype-positive antibody. However, anti-idiotype antibodies do not necessarily react only with idiotype-positive antibodies. Anti-idiotype antibodies raised against antibodies specific for physiological effector molecules (for example, insulin) have been shown to recognize the physiological receptors for the molecule concerned[111–114] even though there is no evidence that these receptors are immunoglobulin related. Anti-idiotype antibodies labelled early haematopoietic cells which were immunoglobulin negative[115] and in another case an antibody to Thy-1 antigen reacted with the TEPC-15 idiotype[116]. Thy-1 is homologous to V domains[117] and its cross-reaction with TEPC-15 myeloma may be a chance event between two related molecules. Finally, a molecule was identified from a T-cell hybridoma which bound azobenzene-arsonate hapten and was recognized by antibodies to an idiotype associated with anti-hapten antibodies. However this was of no relevance to T receptors as the molecule has now been found in the cytoplasm of many cells[118].

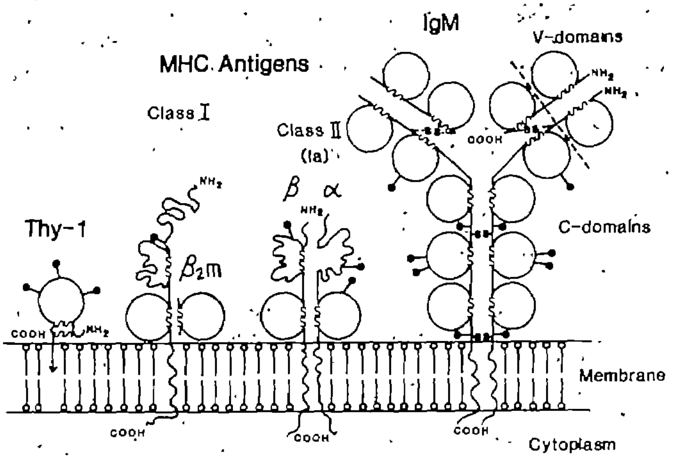

Fig. 3 Immunoglobulin-related molecules found at lymphoid cell surfaces. The diagrams are drawn roughly to scale and represent the polypeptide chains of: Thy-1 differentiation antigen; the major histocompatibility complex (MHC) class I and class II antigens; and IgM. The class I and class II antigens show homology with immunoglobulin C domains. Thy-1 shows homology with V domains. The circles represent immunoglobulin domains and their homologous regions in the other molecules with intra-chain disulphide bonds shown by ⁞ symbols. N-linked carbohydrate structures are shown by ⫯. In the MHC antigens the polymorphic determinants are found mainly in the heavy chains of class I antigens and the β chains of class II antigens. It is highly probable that the class I structure applies to Qa and Tl[145] differentiation antigens as well as to the class I histocompatibility antigens. Qa and Tl have β_2-microglobulin (β_2-m) in their structure and a gene which maps to the *Qa* region and may code for a Qa antigen has been sequenced[146] and shown to have an immunoglobulin-like segment as shown for the large chain of class I antigen. The diagrams are based on: Thy-1, refs 117, 131; MHC antigens, class I, ref. 147; class II, refs 36, 148–150; IgM, refs 1, 2. (Reprinted from ref. 151 with permission of the Biochemical Society.).

Given the above, it is clear that a reaction between an anti-idiotype antibody and a cell or cell product does not provide a definitive assay for the products of antibody V genes. Yet this interpretation has consistently been applied to studies which show that anti-idiotype antibodies can affect helper or suppressor T-cell functions *in vivo* or *in vitro* or react with suppressor factors in ill-defined cell extracts.

In a variety of systems anti-idiotype antibodies can be raised against a related set of antibodies which predominate in the response to a given antigen in a particular mouse strain[119]. The expression of the idiotypes in the antibody response is often linked to the immunoglobulin H-chain constant region allotypes. The anti-idiotype antibodies can stimulate helper or suppressor T lymphocytes specific for the given antigen[40,119–122]. These effects are only seen in the mouse strains which carry the immunoglobulin heavy chain haplotypes which correlate with the appearance of the idiotype in the antibody responses[40,121,122]. One interpretation of these data is that the T cells express the idiotype-positive V domains[40]. However, an alternative is that the idiotype determinants are expressed in non-immunoglobulin framework sequences and that T-cell clones expressing these determinants are selected by regulatory events dependent on the expression of the antibody idiotypes[123]. Until detailed molecular data on the T cell products are available the phenomena cannot be interpreted.

In other experiments suppressor factors in cell extracts have been shown to bind specifically to anti-idiotype antibody columns[34]. Again no molecular interpretation is possible until the factors are characterized in detail. It may not be too long before one has a detailed analysis of suppressor factor specific for keyhole limpet haemocyanin, because the translation from mRNA of products with this activity has recently been described[124]. This should lead to isolation of the suppressor factor genes.

Experiments on idiotypes of T-cell receptors for the major transplantation antigens appeared to hold hopes for molecular analysis. Binz and Wigzell prepared anti-idiotype antibodies against these receptors which labelled the functionally relevant cells and cross-reacted with antibodies of the same specificity[39]. They reported purification of the receptors and prepared rabbit and chicken antibodies against them[125,126]. This was all completed by 1976, yet there has been no further progress and no convincing repeats by other laboratories[127]. It is surprising that the pure receptor has not been used to raise monoclonal antibodies. It seems likely that the situation is not as clear as was at first thought and that difficulties of interpretation apply even though data which appeared decisive were reported.

In our view the idiotype data have been consistently over-interpreted and this criticism can also be made of recent data which claim the identification of allotypic determinants on the constant region of T receptors. Conventional[128] and monoclonal[129,130] alloantibodies have been prepared which react with determinants linked to immunoglobulin H-chain genes and it is claimed that they react with T receptors since the antibodies inhibit a reaction between T cells and antibody idiotypes[128] or react with T suppressor factors[130]. How reliable these systems are for assaying T receptors remains to be seen but it is disappointing that only cytotoxicity analysis, which is notorious for possible artefacts, has so far been used to determine with which cells the antibodies react[129]. Quantitative binding studies using the fluorescence-activated cell sorter have not been carried out

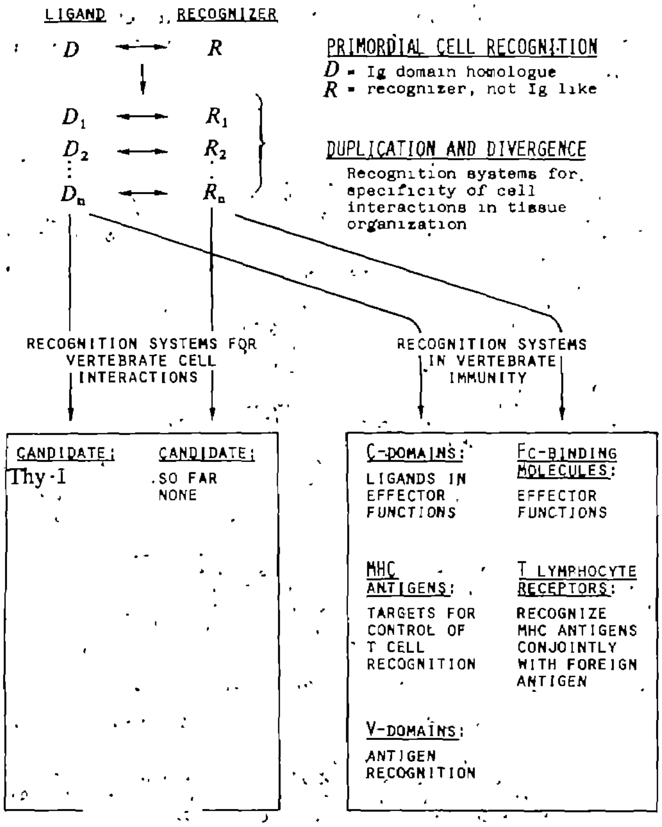
LIGAND
RECOGNIZER
D
R
PRIMORDIAL CELL RECOGNITION
D = Ig domain homologue
R = recognizer, not Ig like
D_1
R_1
D_2
R_2
D_n
R_n
DUPLICATION AND DIVERGENCE
Recognition systems for specificity of cell interactions in tissue organization
RECOGNITION SYSTEMS FOR VERTEBRATE CELL INTERACTIONS
RECOGNITION SYSTEMS IN VERTEBRATE IMMUNITY
CANDIDATE: Thy-1
CANDIDATE: SO FAR NONE
C-DOMAINS: LIGANDS IN EFFECTOR FUNCTIONS
Fc-BINDING MOLECULES: EFFECTOR FUNCTIONS
MHC ANTIGENS: TARGETS FOR CONTROL OF T CELL RECOGNITION
T LYMPHOCYTE RECEPTORS: RECOGNIZE MHC ANTIGENS CONJOINTLY WITH FOREIGN ANTIGEN
V-DOMAINS: ANTIGEN RECOGNITION

Fig. 4 Possible evolution of recognition molecules for cell interactions and immunity.

and it seems premature to claim that the determinants are part of the T receptor constant regions.

Immunoglobulin-related molecules at the cell surface

A surprising number of cell-surface molecules are now known to have segments homologous with immunoglobulin domains. These are illustrated in Fig. 3 and listed in Table 1. It is remarkable that the list includes all the molecules found on lymphoid cells with sufficient known sequence to allow analysis of homologies. It seems likely that more immunoglobulin related membrane molecules will be discovered.

Experiments looking for serological cross-reactions between antibodies and T-cell receptors imply an assumption that a relationship with immunoglobulin will be diagnostic for molecules concerned with immune recognition. The homologies with histocompatibility antigens may be considered consistent with this since histocompatability antigens are involved in the presentation of antigen to T lymphocytes. However, Thy-1 antigen cannot be thought of as having a unique role in immune phenomena since in different species its expression is conserved in neuronal cells and fibroblasts but not in lymphoid tissues[131]. Thy-1 is homologous with V domains[117] and this emphasizes the point that a relationship with immunoglobulin cannot in itself lead to the identification of an antigen receptor.

The genes for the immunoglobulin-related molecules map to six different chromosomes in the mouse (Table 1) and there is no reason why the T-cell antigen receptor genes should be linked to any of these. On the contrary the data suggest they might be quite independently located since there is no linkage between the immunoglobulin genes for H chains and κ and λ L chains.

Alternative possibilities

Given Fig. 1 it seems obvious to say that the T-cell receptor should be related to immunoglobulin as 'everything' else is. Yet this may not be so, and in Fig. 4 we present a proposal for the independent evolution of antibodies and T-cell receptors from the two arms of a primitive recognition system. We suggest that the role of the primordial immunoglobulin domain was to function as a ligand for triggering cell–cell interactions by binding to a recognizer, which was not immunoglobulin-like, on other cells. The Thy-1 glycoprotein may be a contemporary molecule with such a role[132,133]. In evolution, genes for both the primordial domain and its recognizer might have duplicated and diverged to produce a set of molecules for determining the specificity of cell interactions in tissue formation. This system may have given rise to antibodies and histocompatibility antigens at the time of vertebrate evolution. The role of the immunoglobulin Fc domains in triggering effector functions and of the histocompatibility antigens in controlling T-cell recognition would be directly analogous to the triggering role of the primitive set of immunoglobulin-related structures. The Fc-binding molecules of the immune effector systems may have evolved from the recognizer arm of the primitive system, and this may have also given rise to the T-cell receptors.

It could be argued that a distinction between ligand and recognizer in systems like this is semantic and also that both arms of the postulated system could be immunoglobulin-related. Amino acid sequences of the various Fc-binding proteins will show whether or not these constitute an independent family of molecules. Thus far the only relevant protein which has been sequenced in C1q and this shows no homology with immunoglobulin[134].

What next?

We conclude that there is now no good paradigm for thinking about T lymphocyte receptors in molecular terms. Different T-cell clones have different recognition specificities so T receptors must exist but their interaction with antigens is unlike that seen between antibodies and antigens. Somehow there is co-recognition of histocompatibility antigens along with foreign antigen on a presenting cell. The T-cell receptors are unlikely to incorporate any of the gene products seen in antibodies but they may be immunoglobulin-related. However, this does not help greatly in searching for T-cell receptors since many surface molecules are immunoglobulin related.

T-cell receptors can now be sought at either the protein or nucleic acid level. Suppressor T-cell factors are being purified[124,135–137] and their structure will be of interest since they are the only known T-cell products which seem able to interact directly with antigen. However, their combining sites may be quite different from those of helper and cytotoxic T cells which show co-recognition of self-histocompatibility antigen and foreign antigen. Another approach is to search amongst differentiation antigens recognized by monoclonal antibodies for molecules which are candidates for T receptors. A number of antibodies which inhibit or stimulate T lymphocyte responses have been described[138–143]. The molecules they recognize may be receptors for foreign antigen or self-histocompatibility antigen or other regulators of T-cell functions. A similar but possibly more direct approach is to look for monoclonal antibodies which distinguish between T-cell clones and thus perhaps recognize idiotypes of the receptors[144]. At the nucleic acid level characterization of genes which cross-react with immunoglobulin probes may yet be productive and T-cell-specific molecules can be sought at the level of mRNA. In all these approaches the evidence for relevance to T-cell receptors will be weak until sequence data are available. Only then will it be feasible to try and detect in T-cell clones with different recognition specificities molecules of varying sequences in a constant framework. If such molecules were T-cell specific and if antibodies to them inhibited T-cell functions at the level of primary recognition of antigen, we would be confident that the T lymphocyte antigen receptor had, at last, been identified.

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Note added in proof: Mouse monoclonal antibodies specific for common determinants of human V_H labelled a high proportion of B but not T lymphocytes[157]. A molecule which may be mistaken for T-cell immunoglobulin is gp70 of leukaemia viruses, which is immunoprecipitated from extracts of mouse lymphoma cells by chicken anti-mouse immunoglobulin antibody[158].

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ARTICLES

DSDP Hole 504B, the first reference section over 1 km through Layer 2 of the oceanic crust

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Hole 504B provides a reference section for correlation of in situ *petrological and geophysical studies with regional crustal models based on dredged samples and remote surveys; and, at least for the medium-spreading-rate Costa Rica Rift, Hole 504B confirms that the main features of the upper part of an idealized ophiolite sequence occurs in the oceanic crust.*

OUR knowledge of the nature of the ocean crust is drawn from three main sources: (1) studies of ophiolite complexes, (2) surveys using remote geophysical tools such as marine seismic reflection and refraction, magnetics, gravity, and heat flow, and (3) petrological and physical measurements on dredged and cored samples. Pioneers[1,2] recognized early-on that the various rocks dredged from the Mid-Atlantic Ridge were equivalent to the different components of an ophiolite sequence. Several models of the ocean crust have been proposed since which attempted to integrate the various data, but these were usually based on an ideal ophiolite lithostratigraphy (see, for example, refs 3–6). A typical ophiolite complex starts with a pelagic sediment cover resting on effusive pillow basalts, which grade downward into a basaltic dyke complex. This then grades into a plutonic sequence of gabbros and ultramafic cumulates. The lowermost member of the ophiolite sequence is made up of tectonized and metamorphosed ultramafics (ref. 7 and refs therein).

In seismic models of the oceanic crust[8–10], fresh or 'weathered' basaltic pillows and flows (Layer 2A) rest under pelagic sediments (Layer 1) and on metamorphosed pillows (Layer 2B), which in turn rest on metamorphosed intrusive dykes (Layer 2C). Originally called the 'volcanic layer'[2], Layer 2 is on average 2.5 km thick, but its thickness is extremely variable. On the other hand, the underlying 'oceanic layer' (Layer 3) is remarkably uniform in thickness, averaging ~4.5 km, and is thought to be made up of gabbros and more or less serpentinized ultramafics.

The total, seismically-determined thickness of the oceanic crust generally ranges from 5 to 7 km, whereas the total thicknesses of most ophiolite complexes do not exceed 4 km (ref. 7). This, coupled with chemical evidence, suggests that various ophiolites probably correspond to different types of tectonic environments. Thus, one is led to question the validity of using ophiolites as models of ocean crust. Another discrepancy between the ophiolite model and ocean crust is the rarity of dredged and drilled oceanic rocks which can be ascribed to a dyke complex. Finally, from published ophiolite studies one is led to believe that basaltic rocks from ophiolites generally display an increasing degree of pervasive alteration ranging from zeolite facies at the top of the pillow lava to greenschist facies at the bottom of this sequence, to amphibolite facies in the dyke complex. However, the rare detailed petrological studies of metamorphic rocks from ophiolite complexes[11] indicate polyphase and more localized metamorphism.

Leg 83 of the Deep Sea Drilling Project (DSDP) has established the most complete reference section to date through the upper oceanic crust, by deepening Hole 504B in the eastern equatorial Pacific to a total depth of 1,350 m below the sea floor (BSF), 1,075.5 m into basement (Fig. 1). Hole 504B had been drilled to 836-m BSF 2 yr previously during Legs 69 and 70, for the purposes of investigating young, geothermally-active ocean crust[12,13]. In November–December of 1983, Leg 83 re-entered the hole, cored 514 m deeper into basement, and completed an extensive suite of geophysical experiments.

Hole 504B is located in 3,460 m water, 201 km south of the Costa Rica Rift (CRR), the easternmost arm of the Galapagos Spreading Center (Fig. 1). Site 504 is located on magnetic anomaly 3′, with an estimated age of 6.2 Myr. Young crust on the southern flank of the CRR passes through the equatorial high productivity zone, with a sedimentation rate of 50 m Myr^{-1}. The 274.5 m of sediment at Hole 504B are dominantly siliceous nannofossil oozes, of late Pliocene age near basement contact. The youngest chert yet found in the oceans occurs at the base of the sediments[12]. This chert is thought to have been formed by the high temperature within the thick pile of sediments overlying young oceanic crust[14].

Geothermal setting and temperature measurements

In the vicinity of Site 504, the thick sediment has formed an effectively impermeable seal to convective heat loss from the cooling plate. This is demonstrated both by the pattern of surface heat flow measurements[15,16], and by downhole temperatures[12,17,18], all of which indicate surface heat flow in agreement with conductive plate cooling predictions of 190–

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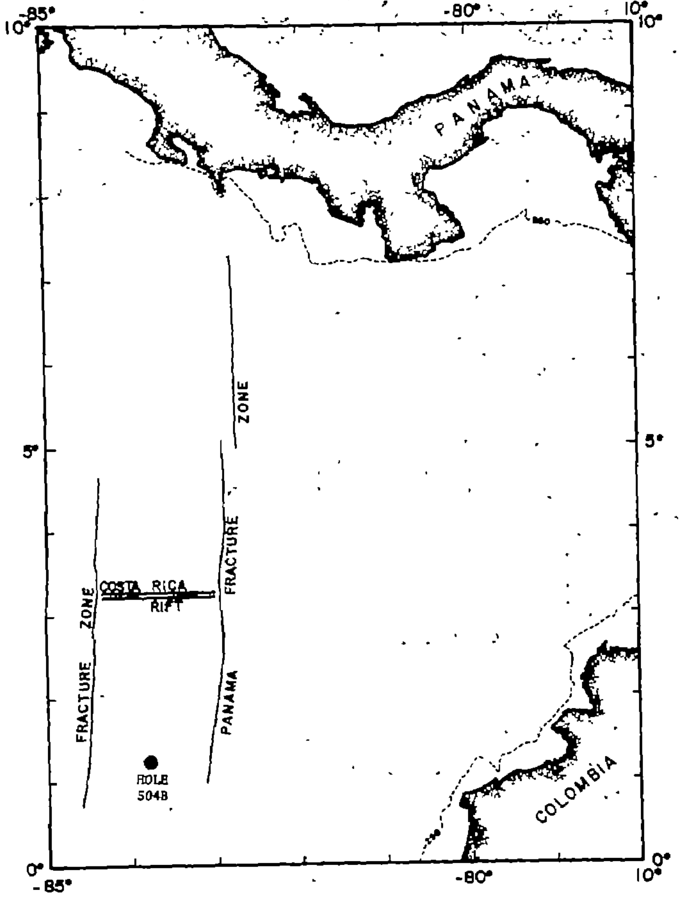

Fig. 1 Location of Hole 504B.

200 mW m^{-2}. Closer to the spreading axis, where the sediment cover is thinner and the basement topography is considerably rougher than at Site 504, surface heat flow values are much less than predicted, presumably due to hydrothermal convection[16].

Downhole temperatures measured on Legs 69, 70 and 83 indicate that an equilibrium, predominantly conductive geothermal gradient holds to the full Hole 504B depth (Fig. 2). However, after Leg 69 drilling punctured the sediment seal, borehole temperatures to a depth of ~360-m BSF were strongly depressed below values measured in the sediments. This was interpreted to indicate flow down the hole of ocean bottom water into the uppermost ~100 m of basement, at a December 1979 rate of 6,000 l h^{-1} (ref. 17), decreasing to ~1,500 l h^{-1} in November 1981[18]. This drawdown is convection forced by basement pore fluid underpressures, which have been attributed to hydrothermal circulation in the basement[19]. The basement underpressure originally measured on Leg 69[19] must have significantly rebounded towards the equilibrium hydrostatic pressure after 2 yr of mass flux down the hole into the formation: a mass of seawater of the order of 50×10^6 kg.

Lithostratigraphy

The combined drilling efforts of Legs 69, 70 and 83 have resulted in the penetration in Hole 504B of 1,075.5 m of oceanic basement, with a total recovery of 224 m, corresponding to an average of 20.8%. The lithostratigraphy is shown schematically in Fig. 3 and is summarized below. The upper 575 m of basement consists of intercalated pillow lavas, pillow breccias, hyaloclastites, minor flows, and localized flow and tectonic breccias.

The upper zone is underlain by a 209 m transition zone consisting of pillows, minor flows, and dykes. The upper boundary of the transition zone has been placed at a depth of 846-m BSF, where the first three dykes were encountered within 5 m of each other. Pillow sequences are more abundant, continuous and heavily brecciated at the top of the transition zone than at the bottom. Fracturing and brecciation are particularly extensive from 911 to 929-m BSF within a large pillow sequence where a mineralized stockwork occurs. Towards the base of the transition zone the pillow units become thinner, and massive units (with a uniform, medium coarse-grained texture, lacking chilled margins) and dykes progressively increase in frequency. The massive units cannot be identified explicitly as dykes because they lack chilled margins.

Dykes were identified by the intrusive nature of the contacts determined in hand specimens and thin sections. Macroscopically, the contacts range from brecciated to razor sharp. In contrast to chilled pillow rims, glass is never encountered in dyke margins and extensive quench morphologies are rare. Dyke margins are best described as having a dense cryptocrystalline to very fine-grained equigranular texture (crystals <0.004 mm in size). Microlites and microphenocrysts are

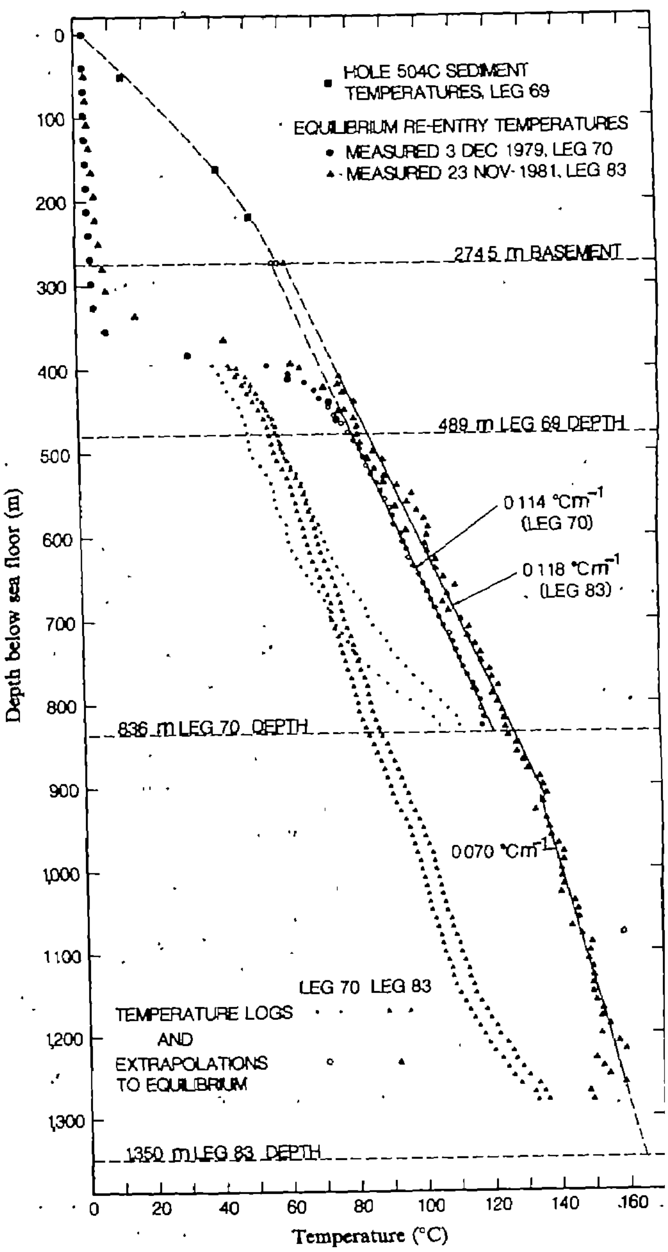

Fig. 2 Borehole temperatures measured on Legs 69, 70, and 83. Equilibrium re-entry temperatures (circles for Leg 70 and crosses for Leg 83) indicate drawdown of bottom water into the upper ~100 m of basement, at a decaying rate. Below this aquifer, extrapolation of temperature logs to equilibrium indicates a conductive heat flow of ~190 mW m^{-2} (from ref. 18.)

nearly always preferentially aligned parallel to the contacts and disaggregated material from the host basalt is also commonly observed at the margins of the intrusive material.

The last recovered pillow was identified at 1,055-m BSF; this depth was chosen by the shipboard petrographers as the boundary of an underlying zone composed predominantly of massive units and dykes in which no pillows were recognized. This unit is interpreted as a sheeted dyke complex, as predicted from ophiolite models of the oceanic crust. The dykes were recovered with one, and rarely two, chilled margins, most of which were steeply inclined (50–60°) to near vertical. Some of these chilled margins are highly brecciated, possibly as the result of forceful dyke intrusions. However, this zone as a whole is far less brecciated than those overlying it. The host material against which the dykes were chilled is always fine to very fine-grained. In contrast, basalts from the inner portions of dykes as a whole tend to have among the coarsest grain sizes recovered (medium coarse-grained, subophitic textures). Although the data are not yet sufficient for a convincing argument, this observation may indicate that dykes tend to intrude predominantly at the margins of previous dykes. This appears to be consistent with observations of one-way chilling documented in ophiolites[20]. Most of the basalts recovered from the lower zone are regarded as massive. Many of these exhibit significant grain-size variation; that is, fining towards one or both of their implied margins. Such massive units are probably dykes, although (per our definition of a massive unit) no chilled margins were recovered. However, the remainder of the basalts maintained a constant grain-size over the recovered interval and we can only speculate about their mode of emplacement.

The lavas in the uppermost part of Hole 504B are predominantly fine- to medium-grained, olivine + plagioclase ± clinopyroxene ± spinel phyric basalts; aphyric lavas become progressively more common with depth. These basalts can be divided into four petrographic categories on the basis of the phenocryst contents: (1) olivine–plagioclase–clinopyroxene phyric (~29% of the total recovery); (2) olivine–plagioclase phyric (13%); (3) variable olivine–plagioclase–clinopyroxene phyric with accessory chrome spinel (19%); (4) aphyric (40%).

Basement geochemistry

The least-altered basaltic rocks recovered from Hole 504B are remarkably uniform in their vajor oxide composition. Moreover, no chemical distinction can be made amongst the various petrographic or lithological types. Within the 1,075.5 m basement section, only three lithological units, 14, 18 and 2 m thick, differ significantly from all the other units. These basalts are characterized by higher TiO_2 and P_2O_5 concentrations (1.2–1.4% and 0.14–0.20%, respectively) and by an enrichment of LIL-elements, whereas most of the Legs 69 and 70 basalts are strongly LIL-element depleted[21].

A general characteristic of the rocks cored during Leg 83 is their high MgO (up to 9.78%) and their very low K_2O content, which is <0.02%. No systematic downhole variation was observed for the major oxides except MgO and K_2O (see section on alteration). However, there are some minor but irregular variations with depth, which are related to specific lithological units and indicate that limited shallow-depth fractionation processes were operative. The oxide means obtained on Leg 83 rocks are almost identical to those found in Legs 69/70 basalts and their confidence limits in all cases overlap. On a dry weight normalized basis, most rocks analysed vary within the following limits: SiO_2, 49.9% ± 0.9; TiO_2, 0.92 ± 0.1; Al_2O_3, 15.7 ± 0.8; FeO_t, 9.05 ± 0.5; MgO, 8.60 ± 0.6; CaO, 12.9 ± 0.3; K_2O, 0.20 ± 0.1 (Leg 69 section), 0.07 ± 0.04 (Leg 70 section) and 0.02 (Leg 83 section); P_2O_5, 0.08 ± 0.02.

Similar ranges of variation were also obtained on fresh basaltic glasses from the upper part of the hole drilled during Legs 69 and 70. These results suggest that the whole rock analyses (with the possible exception of MgO and K_2O) reflect the primary composition of the rocks and might represent magma compositions. Judging from the high mg values (where $mg \equiv Mg^{2+}/(Mg^{2+} + Fe^{2+})$), which range from 0.62 to 0.70 and average at 0.67, these magmas were rather primitive or unevolved, and had experienced only a small degree of crystal fractionation before their eruption or intrusion. This indicates either a short residence time within the magma chamber and/or the existence of a large magma chamber which was steadily replenished by the same mantle source.

Assuming that the least altered basalts of Leg 83 have similar trace element composition as those from Legs 69/70, it can be concluded that the magmas were derived from a LIL-element depleted mantle source. As a working hypothesis we assume that basalts of the three exceptional units were generated within very small 'pockets' of less depleted mantle material residing below the magma chamber.

Alteration

Alteration of basalts in Hole 504B can be divided into different depth zones based on the presence of various secondary mineral assemblages (Fig. 4). Secondary mineral occurrences from 275 to 585-m BSF are similar to those commonly observed in drilled submarine basalts altered at low temperatures ('submarine weathering'; refs 22–25). Alteration of the rocks in this interval is interpreted to be the result of superimposed stages of oxic alteration ('iddingsite' and celadonite–nontronite mixtures replacing olivine and filling voids in alteration haloes around cracks) and sub- to anoxic alteration (saponite replacing olivine and filling voids throughout the rocks).

The interval from 528 to 572-m BSF is characterized by the presence of Na–Ca zeolites in relatively frequent thick veins and replacing plagioclase in 1-cm wide alteration haloes around these veins. These zeolite veins and associated wallrock alteration in this zone are interpreted to be the result of a later localized stage of hydrothermal alteration superimposed on the previous alteration at lower temperature[26].

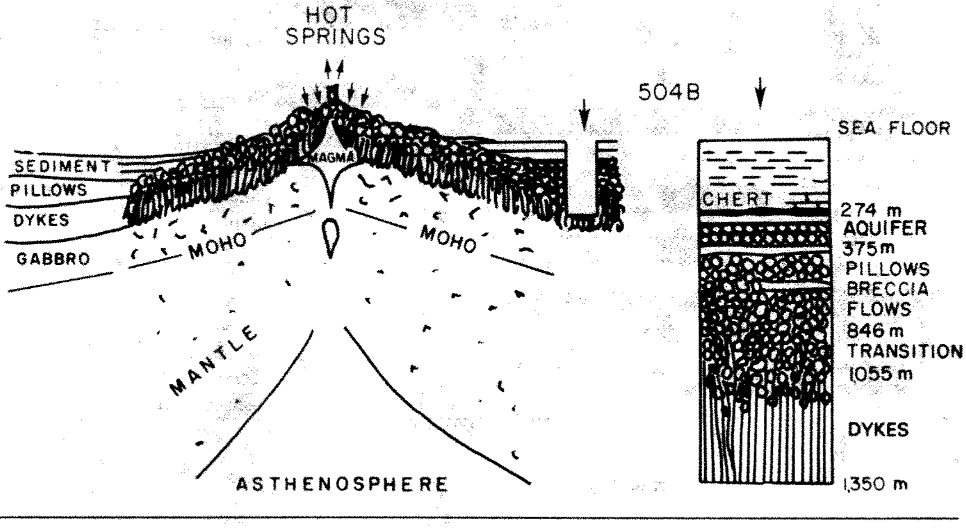

Fig. 3 Schematic cross-section of the Costa Rica Rift, with a highly generalized lithostratigraphy based on Hole 504B recovery and Leg 83 geophysical results.

From 585 to 889-m BSF, alteration is characterized mainly by the occurrence of saponite replacing olivine and filling voids. Secondary minerals in this interval shown in Fig. 4 also occur in veins, with quartz and pyrite abundances generally increasing with depth. Based on mineralogical data and bulk rock oxygen and stontium isotopic data, alteration reactions are interpreted to have occurred in sub- to anoxic conditions either with seawater at low water to rock ratios, or with seawater-derived fluids that had previously reacted with basalt[26,27]. This stage resulted in the bulk of the alteration observed, characterized mostly by the formation of smectite. Preliminary oxygen isotopic work on isolated smectite and anhydrite suggests that alteration occurred at 60–110 °C (refs 26, 28).

From 889 to 1,350-m BSF, olivine is generally totally replaced by, and interstitial areas filled with chlorite, although smectite or smectite–chlorite mixtures occasionally occur. Titanomagnetite is partly replaced by sphene, and plagioclase is partly replaced by albite, chlorite and minor laumontite. Actinolite occurs as alteration rims of clinopyroxene below 968-m BSF. The above minerals also occur in veins and cementing breccias, whereas the other secondary minerals in this interval (Fig. 4) only occur in veins or breccias.

Based on vein relationships, experimental data[29], and analogy with Icelandic geothermal fields[30,31], alteration from 889 to 1,350-m BSF is interpreted to have occurred in at least two stages at successively decreasing temperatures. The first stage resulted in the formation of actinolite, chlorite, sphene, albite, pyrite, and possibly epidote and quartz. Temperatures were at least 230 °C, and were >280 °C where actinolite formed (below 968-m BSF). During the second stage, laumonite, scolecite, pyrite, minor amounts of calcite, and possibly chlorite and prehnite formed at temperatures <235 °C. The occasional occurrences of smectite below 889-m BSF remain a problem, however, and may indicate either a lower temperature of alteration superimposed on a greenschist facies stage, or that smectite formed metastably at high temperatures.

A stockwork consisting of abundant veins of chlorite, laumontite, quartz, minor talc, sphalerite, chalcopyrite, and common large crystals (up to 1 cm) of pyrite in highly fractured pillow basalts occurs from 911 to 929-m BSF. Alteration in this stockwork occurred in two stages, similar to those listed above. The abundant sulphides in this zone can be explained either by mixing of a metal, S, and Si-rich hydrothermal fluid with seawater, as is suggested to occur at the Galapagos Spreading Center[32], or by a drop in temperature and/or pressure of this solution due to its rise and loss of heat to wall-rock.

Between 1,125 and 1,280-m BSF the rocks are generally less extensively altered and contain fewer and thinner veins than the overlying rocks. Olivine is completely altered to talc, magnetite, and smectite, whereas other igneous minerals are only very slightly altered. The smaller extent of alteration of these rocks may be due to the massive nature of the rocks and the scarcity of veins and cracks which would result in low permeability and restricted circulation of fluids.

From 1,182 to 1,350-m BSF anhydrite occurs in veins, and is interpreted to be a late alteration product. Anhydrite may have formed from heated Ca-enriched seawater in the current thermal regime, as predicted by experimental seawater–basalt reactions[33], or by mixing of hydrothermal fluids with seawater, as at 21 °N on the East Pacific Rise[34].

Below 1,280-m BSF basalts are more extensively altered than those in the overlying interval (1,205–1,280-m BSF), and the lowermost rocks recovered are among the most extensively altered.

There is a systematic but slight MgO increase and an associated K_2O decrease with depth as a result of hydrothermal alteration. H_2O+ is generally $<1.0\%$ in the least altered rocks from Legs 69 and 70 part of the hole, but shows a maximum (up to 3.4%) between 980 and 1,075-m BSF, coinciding with a zone of extensive mineral replacement; it then drops again towards the bottom of the hole. CO_2 decreases from top (0.1–0.4%) to bottom of the hole, where it is $<0.02\%$ in most samples. Note that only the least altered rock samples were analysed, so the chemical changes due to alteration are minimal.

Physical changes down the hole

Leg 83 also conducted the most extensive set of geophysical logs and experiments yet in the Deep Sea Drilling Project (Fig. 5), including packer flow tests, large-scale resistivity[35], borehole televiewer and multichannel sonic logging.

Geophysically, the upper oceanic crust sampled at Hole 504B consists of three distinct units. The upper ~100 m of basement is an aquifer of rubbly pillow basalts, breccia zones and a few massive flows. The entire section is fractured but not as extensively as deeper in the hole. Density, thermal conductivity, velocity (both P- and S-waves) and electrical resistivity are all low. Attenuation of seismic body waves along the wellbore and neutron porosity are high, and measured bulk permeability values are as high as those of a good producing oil sand (~60 millidarcies, md). An 'alteration analysis' carried out by cross-correlation of hydrogen-ion-sensitive neutron porosity versus porosity-sensitive γ-ray density logs corroborates Leg 69 core

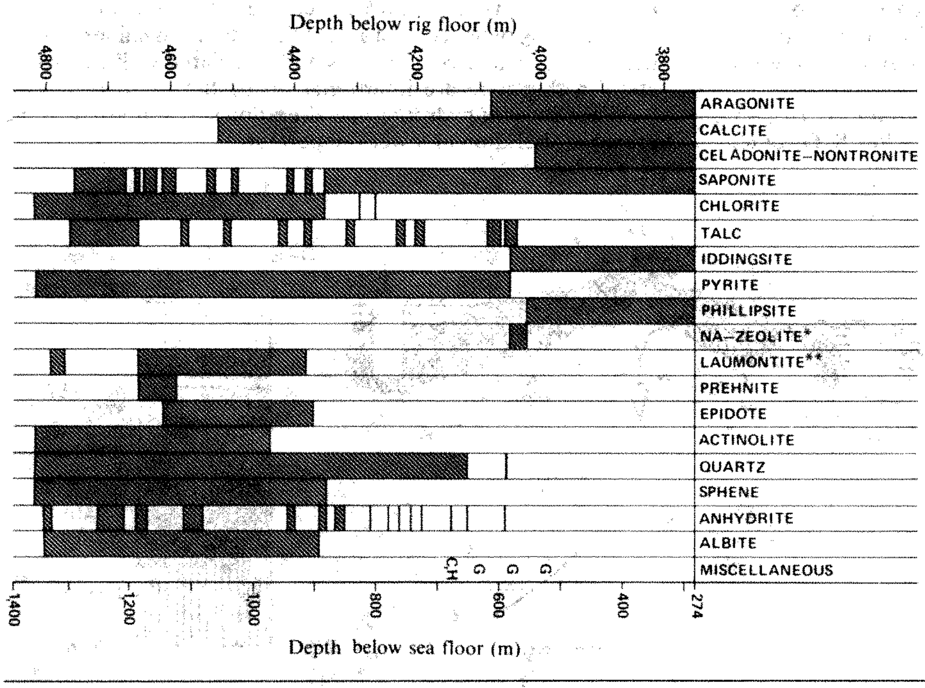

Fig. 4 Occurrences of alteration minerals in basalts recovered from Hole 504B. *, Natrolite, mesolite, thomsonite, analcite, apophyllite; **, including rate scolecite; G, gyrolite; C, chabazite; H, heulandite.

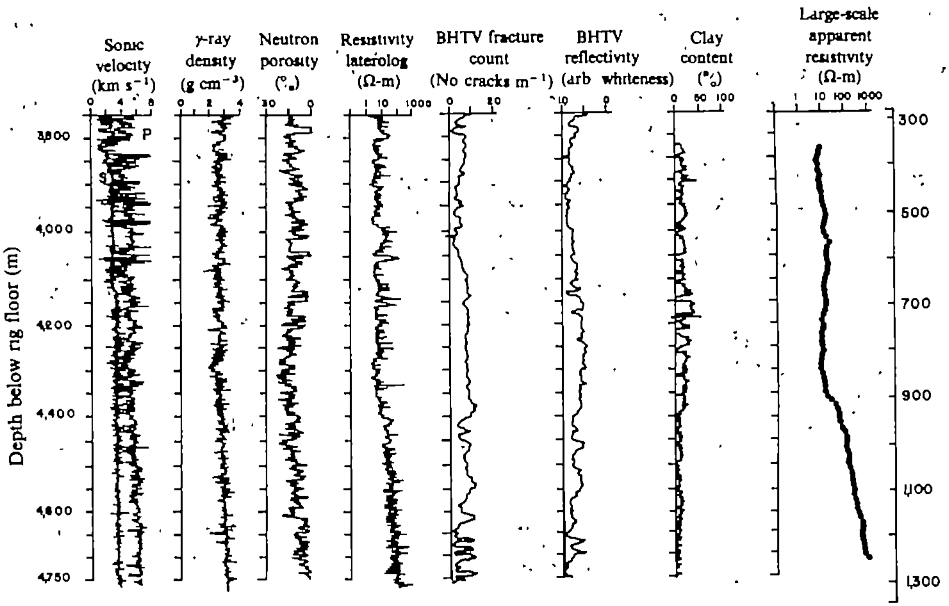

Fig. 5 Geophysical logs obtained from Hole 504B on Leg 83.

analyses[13] that show only moderate crack-filling by OH⁻ bearing alteration products such as clays and zeolites. Fractures thus appear to be largely open and filled with seawater[36]. Underpressures then turned this upper crust into an active aquifer system when the drill bit penetrated the chert and sedimentary hydraulic lid over this formation. We interpret this zone to be seismic Layer 2A.

From ~100 to 650 m into the basement (375–925-m BSF), increasing gradients in seismic P- and S-wave velocities, electrical resistivity and density, and a decreasing gradient in porosity are observed. Permeability at 475-m BSF has dropped to 4 md. The log cross-correlation alteration analysis shows a steadily increasing bulk content of hydrated minerals, peaking in a zone at 911–929-m BSF, the highly altered, sulphide-rich stockwork.

Borehole televiewer records show a steadily increasing content of fractures (almost all subhorizontal) and a decreasing wellbore reflectivity as the Layer 2A zone of large pillows appears to be replaced by much more extensive brecciation, smaller diameter pillows and fewer massive flows. Shipboard analysis of the cores recovered from this zone could not confirm the decrease in diameter of the pillows as observed in the BHTV records. We interpret this zone to be seismic Layer 2B. Relatively higher seismic velocity seems to be directly related to alteration products filling fractures and voids in this pillow unit[36]. Thermal conductivity, and magnetic susceptibility and intensity remain constant through this layer. Towards the bottom of Layer 2B, the first dykes are encountered.

Then, at about the depth of the stockwork, all of the physical properties change (Fig. 5). From 875 to 925-m BSF, electrical resistivity, density, and seismic velocity all show a slope change to a higher gradient. Porosity drops; sample thermal conductivities show a stepwise increase of ~25%. Magnetic intensity and susceptibility drop as most titanomagnetite is altered to sphene, but this drop is observed above the level where all other physical properties change. The borehole televiewer shows a drop in the intensity of fracturing and a rise in wellbore reflectivity. The log 'alteration analysis' shows a drop in the bulk content of hydrated minerals at this depth also. Permeability is 0.1 md over the lower 750 m of the hole, a drop of nearly three orders of magnitude. We interpret this to be the transition from seismic Layer 2B to 2C. Below, an increasing number of dykes leads into a sheeted dyke complex towards the bottom of the hole.

At 1,075-m BSF, magnetic intensity and susceptibility increase again as alteration pervasiveness subsides and more titanomagnetite remains intact.

The measured permeability decreases below Layer 2A much more abruptly than was previously expected, because alteration has plugged the plumbing in Layer 2B. The primary porosity of the rocks forming Layer 2C was already very low before alteration locally reduced it further. We cannot determine whether this permeability stratification has existed since the ridge axis or whether Layer 2A is 'disappearing' upwards as alteration products progressively replace seawater in fractures and pore spaces of the aging upper crust. If the former is the case, it suggests that much shallower, more horizontally-vigorous hydrothermal convection occurs on mid-ocean ridges than was previously thought.

Conclusions

Leg 83 of the DSDP has established the most complete reference section to date through the upper oceanic crust by deepening Hole 504B to a total depth of 1,350 m below the seafloor, or 1,075.5 m into basement. A maximum temperature of 135 °C was measured at the bottom of the hole, estimated to reach ~165 °C after the disturbance due to drilling has decayed. The 274.5-m thick chert-based sediment cover has sealed the crust to convective heat loss, so that if the crust at Site 504 is assumed to have aged through an earlier, hydrothermally-driven cooling phase, then crustal temperatures must have re-equilibrated upwards to present values as the sediment cover sealed off the basement.

The upper ~100 m of the basement consists of a relatively permeable (~60 md) aquifer made up of pillow lavas and breccias, and a few massive flows. Fractures appear to be open and filled with seawater. This is thought to be seismic Layer 2A. From 375 to 850-m BSF more pillow lavas, breccias, and minor massive flows of seismic Layer 2B were encountered, while the frequent fractures in this interval are filled mainly with smectite. The interval 846–1,055-m BSF corresponds to a transition zone with decreasing frequency of pillow lava recovery with depth and correlative increasing dyke recovery. A mineralized stockwork with Fe, Zn and Cu sulphides in laumontite, calcite, quartz, and chlorite veins was recovered in an extensively fractured and brecciated pillow lava sequence within this zone and may be the result of subsurface mixing of hydrothermal fluids with seawater.

The interval from 1,055-m BSF to the bottom of the hole corresponds to part of a sheeted dyke complex, seismic Layer 2C. Just below the mineralized stockwork, a major increase in density, thermal conductivity, velocity (both P- and S-waves) and electrical resistivity occurs. Conversely, the degree of fracturing, porosity and permeability decrease, the latter by nearly three orders of magnitude compared with the overlying formation. Magnetic susceptibility and intensity generally correlate with the extent of alteration of titanomagnetite.

The Layer 2B–2C transition based on physical properties is much sharper than the lithological transition from extrusive rocks to dyke complex (that is, ~50 m compared with ~209 m, respectively). Moreover the boundaries among the various types of alteration zones with depth do not coincide with any lithostratigraphic or physical property limits.

From the sediment–basement interface down to 585-m BSF (representing Layers 2A and 2B), basement alteration resulted from superimposed stages of sub- to anoxic and oxic processes, the latter at relatively high water–rock ratios. These stages resulted in the formation of celadonite–nontronite, 'iddingsite', pervasive saponite, and, finally, phillipsite and Ca-carbonates (aragonite, calcite). These processes and products are, on the whole, similar to 'sea floor weathering' observed in other DSDP holes. Veins of saponite and Na–Ca zeolites between 528 and 570-m BSF represent a localized later stage of superimposed hydrothermal alteration. Below 585-m BSF down to ~900-m BSF where greenschist-facies minerals appear, only sub- to anoxic alteration at low water–rock ratios has occurred.

Alteration of basalts below 889-m BSF is interpreted to be the result of at least two stages of alteration: (1) earlier formation of actinolite, chlorite, sphene, albite, pyrite, epidote and quartz at temperatures of at least 230 °C, and >280 °C where actinolite is present (below 969-m BSF); followed by (2) later formation of laumontite, scolecite, pyrite, minor amounts of calcite, and possibly chlorite and prehnite at temperatures <235 °C. Rocks in the interval 1,205–1,280-m BSF are much less extensively altered than over- and underlying rocks, however, due to locally lower permeabilities.

We thank Captain Dill and the crew and technical staff of the DV *Glomar Challenger* for their expertise, especially G. Norrie, A. C. Wheeler, and the drilling crew, who saved the hole on several occasions, leaving it open for possible future re-entry and deeper drilling.

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In situ electrical resistivity and bulk porosity of the oceanic crust Costa Rica Rift

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In situ electrical resistivity was measured in DSDP Hole 504B to a depth of 1,013 m into oceanic basement. Apparent resistivities are about 10 Ω m *in about 600 m of pillow lavas, sharply increasing to nearly 1,000 Ω m in the underlying dykes. Bulk porosities calculated from resistivities show a distinct layering, apparently corresponding to geophysical layers 2A, 2B and 2C.*

THE circulation of seawater through fresh basalt has a vital role in the development of the chemical and physical nature of the oceanic crust. The modes and effects of circulation are themselves partially controlled by the variable permeability and porosity of the basaltic layer. As these crustal parameters probably depend on irregular fracturing of unknown scale, they cannot be reliably determined from dredged or cored samples. On the contrary, independent determinations of bulk porosity are essential in relating physical properties measured on recovered samples to the formation properties of the crust. Bulk permeability and porosity must be measured *in situ*, preferably from deep boreholes, at averaging scales large enough to escape the disturbance of drilling on the surrounding formation, as well as to include the effects of irregular fracture porosity.

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We report here the results of such an *in situ* experiment, a large-scale resistivity log in the deepest borehole drilled to date into the oceanic crust, Hole 504B of the Deep Sea Drilling Project (DSDP). Over the temperatures encountered in and around the hole (2–165 °C), the electrical conductivity of the pore fluid, essentially seawater, is 1–4 orders of magnitude greater than the intrinsic conductivity of the host basalt. Therefore, although not a direct measurement of porosity, our results were highly sensitive to the bulk porosity of the crust.

Measurement site

Hole 504B is ~200 km south of the Costa Rica Rift (Fig 1), in 6.2 Myr crust under 3,460 m of water. It was cored by three DSDP Legs, 69, 70 (refs 1, 2) and 83 (ref. 3), to a depth of 1,350 m beneath the sea floor, 1,075.5 m into basement (Fig. 2), nearly twice the basement penetration of any other oceanic borehole. Leg 83 clearly penetrated into the sheeted dykes and massive units of layer 2C (ref. 3). Due to the relatively poor core recovery (~20%), good geophysical logs were essential to the interpretation of the section and to relate studies of recovered samples to the bulk properties of the crust.

The 274.5 m of chert-based sediment have sealed the crust at Hole 504B from open circulation of seawater. Surface heat flow measurements[4] and downhole temperatures[5,6] indicate dominantly conductive heat transfer through the drilled section, at a value of 190±10 mW m^{-2}, consistent with predicted plate heat flow. No direct evidence was found for presently active hydrothermal circulation, although a vigorous, pressure-driven[7] drawdown of bottom water commenced when Leg 69 penetrated the sediment seal[5,6]. This drawdown is directed through the cased sediment into the upper ~100 m of basement, and it continues at a decaying rate[3,6]. It has had no discernible effect on the crust deeper than 400 m beneath the sea floor.

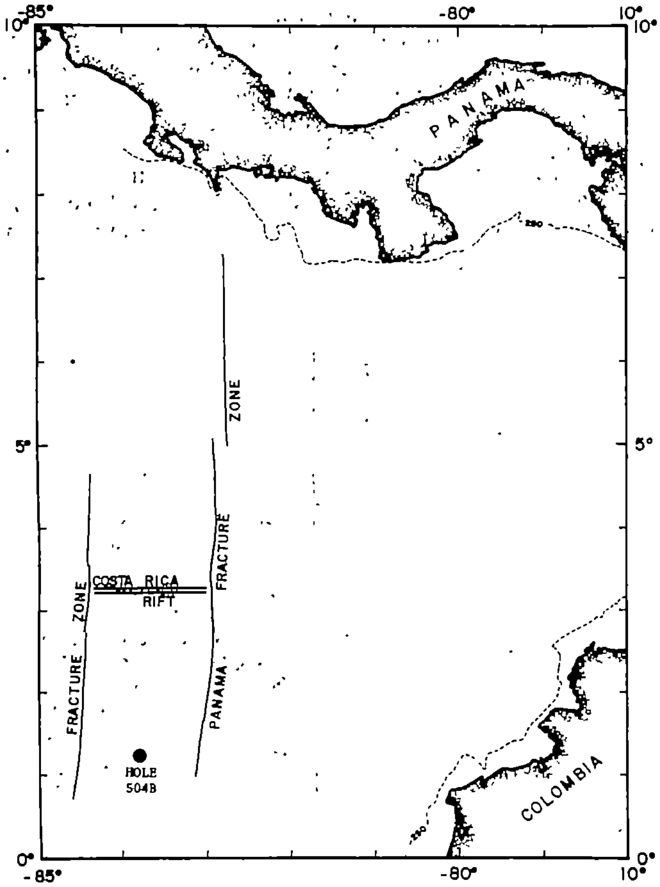

Fig. 1 Location of DSDP Hole 504B in the eastern equatorial Pacific.

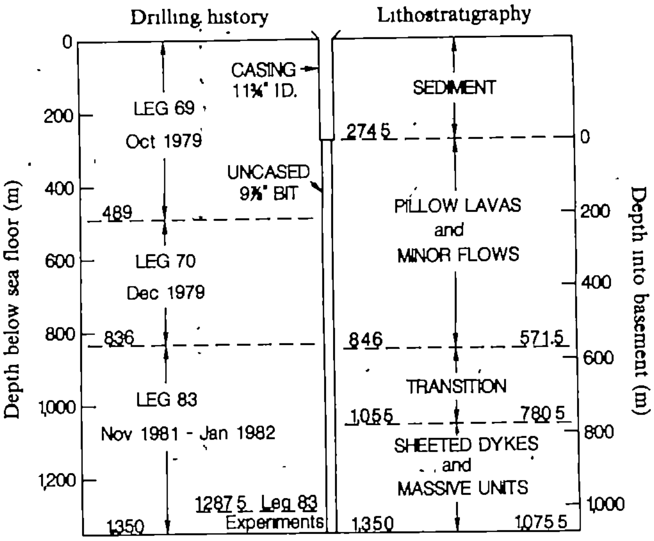

Fig. 2 Hole 504B drilling history (left) and generalized lithostratigraphy (right). The latter is based only on the ~20% core recovery[3].

Experimental procedure

The large-scale electrical resistivity experiment was first deployed on Leg 60 by Francis (see ref. 8 for details of the method). It was run twice in Hole 504B, first during Leg 70 to a depth of 836 m (ref. 9), and again during Leg 83 to 1,287.5 m. The experiment is a simple, long-spaced, d.c. resistivity log, for which the downhole configurations are shown in Fig. 3. A large current is passed from the ship down the armoured logging cable, through an insulated cable to a steel sinker-bar/current-electrode. It then passes through the formation and sea back to the ship. Despite the high conductivity of seawater, the long, narrow seawater return path up the borehole is relatively resistive (~5 kΩ km^{-1}), so that the current may be taken to return through the formation[8]. (Nor do the casing or armoured logging cable shunt the current return path[8].) Due to the resistivity of the formation, a potential gradient is set up in the hole, and it is sampled with several non-polarizing Ag/AgCl electrodes.

The experiment was run at discrete, stationary intervals: at each level, current was passed for about 10 s each under positive and reversed voltage. This allowed removal by averaging of any biases between electrodes due to possible ambient potential gradients in the hole. Currents and potential differences were measured to an estimated 3–5% accuracy on Leg 70, and to 1–3% accuracy with new equipment on Leg 83. On Leg 70, a 500-V power supply passed currents of ~6 A into the formation, and three potential electrodes were spaced 45, 91 and 183 m above the current source (Fig. 3). Potential differences between X and Y electrodes and Y and Z electrodes were recorded and the experiment was run at 25-m intervals. During Leg 83, a 300-V power supply was used, currents ranged over 3.5–4.3 A, and four potential electrodes were spaced 10, 20, 40 and 80 m above the current source. The hole was logged at 10-m intervals, with four potential differences recorded: 1–2, 2–3, 3–4 and 1–4. This redundancy showed excellent Leg 83 data quality: at every level, the sum of the smaller interval voltages duplicated the overall voltage to well within 1%. Although the hole was considerably deeper on Leg 83, a shorter electrode spacing was chosen, partly because the high temperatures in the hole were a threat (unrealized) to melt the insulated cable, and partly to bridge the range of resolution to the much shorter-spaced, focused, commercial resistivity logs also run.

The basic parameters measured were the potential differences between electrodes due to a known power source. Of the nine possible electrode pairs, potential differences were measured for six, and were obtained arithmetically for the other three. Measured voltages were in the 5–50 mV range in the pillow

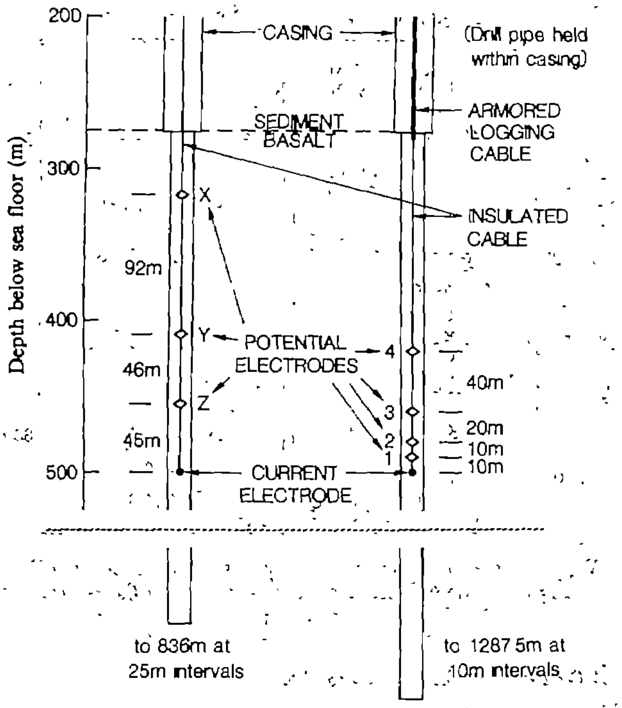

Fig. 3 Electrode configurations for large-scale resistivity experiments in Hole 504B.

lavas logged on Leg 70; when the dykes were logged on Leg 83, voltage differences of 10 mV–10 V were recorded. A 300-m section was repeat-logged on Leg 83; all measured voltages duplicated within 1%.

Apparent resistivity

For a real Earth, with a resistivity varying with depth (and possibly laterally as well), our measured voltage differences are related to the resistivity structure in a complicated, nonlinear fashion. The response of each electrode pair is a function of both the surrounding resistive Earth and of the spacings among potential and current electrodes[10]. In effect each electrode pair averages the Earth resistivity over a unique radial and vertical scale. We approximated true resistivities by converting voltage differences to apparent resistivities (ρ_a), calculated assuming a homogeneous Earth resistivity. With a zero potential assigned to the sea floor, and a current return at the ship, apparent resistivities were obtained by[8]:

$$\rho_a=\frac{2\pi\Delta V}{I}\left\{\frac{z_2}{h^2-z_2^2}-\frac{z_1}{h^2-z_1^2}\right\}^{-1}$$

with h being the depth beneath the seafloor of current source I, and z_1, and z_2 the depths of potential electrodes sensing the voltage difference $\Delta V(h>z_2>z_1)$. Since the potential gradient was more sensitive to resistivity variations where the current density was greater, that is closer to the current source, the depths assigned to apparent resistivities were those of the deeper potential electrode.

The electrode spacings were large enough relative to the hole diameter that no geometric borehole correction was necessary to the measurements. Moreover the large-scale experiment investigated far enough into the formation (~5–100 m) to escape the effects of the disturbed zone near the borehole, where temperature, pressure and porosity may have been unrepresentative of *in situ* conditions. We thus interpret our apparent resistivities as radially and vertically averaged *in situ* resistivities, with the poorly-determined averaging scales dependent on the true resistivity structure.

Figure 4 shows apparent resistivities calculated using five of the nine possible electrode pairs. These five pairs shared the same equidimensional ratios of spacings among the current and two potential electrodes (Fig. 3). Thus their averaging characteristics were similar, except that the longer arrays averaged farther into the formation. Indeed the five curves in Fig. 4 are increasingly smoothed, left to right, with increasing array length. The greater detail in apparent resistivities from the shorter-spaced Leg 83 electrode pairs probably reflects better resolution of small-scale vertical variations in resistivity; the smoother resistivities from the longer-spaced electrode pairs are probably more representative of the bulk properties of the crust.

The apparent resistivities in Fig. 4 are all consistent with bulk crustal resistivities on the order of 10 Ω m through the ~600 m of pillow lavas cored in Hole 504B. Below this level apparent resistivities increase sharply, approaching 1,000 Ω m in the sheeted dykes and massive units of layer 2C. As occurred with all other geophysical measurements in the hole, the transition in apparent resistivities is much sharper than the lithostratigraphical transition zone of ~209 m (Fig. 2) determined from

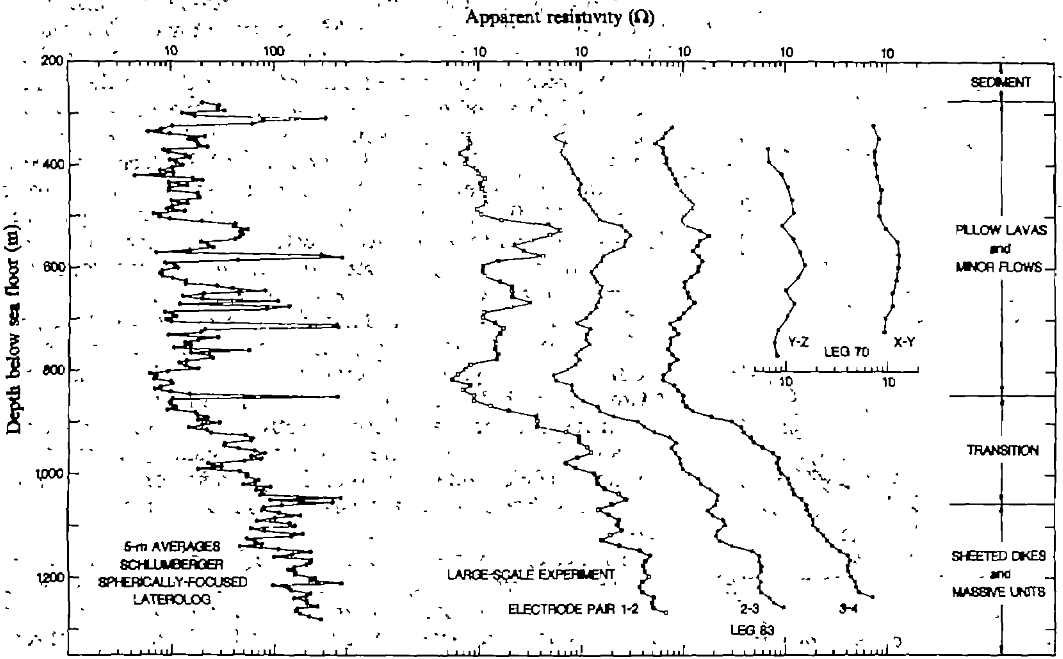

Fig. 4 (Left to right) 5-m averages of borehole-corrected resistivities measured by Schlumberger logging tool, five sets of apparent resistivities from the large scale experiment, and generalized lithostratigraphy of Hole 504B. Note logarithmic scales for resistivities, which are near 10 Ω m in the upper basement and approach 1,000 Ω m in the deepest dykes.

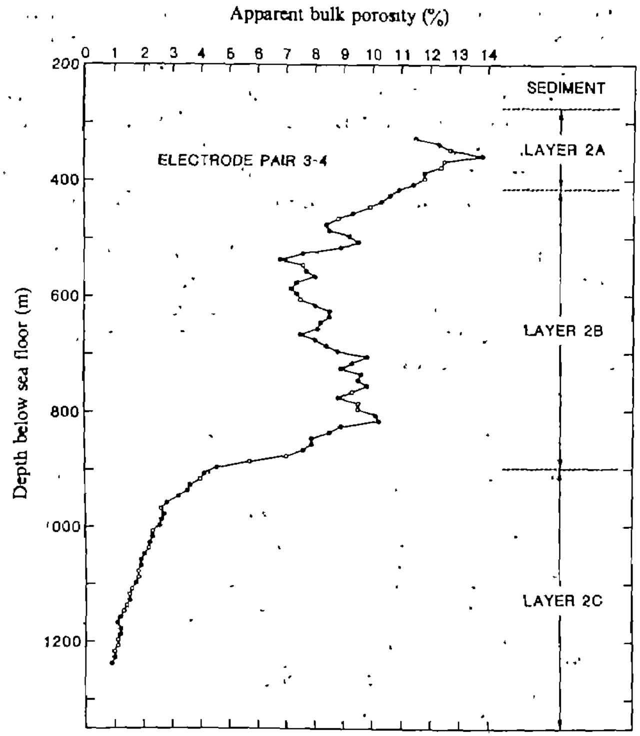

Fig. 5 Apparent bulk porosities calculated from apparent resistivities determined from electrode pair 3–4, using Archie's law with a temperature-corrected seawater resistivity: $\rho_a = \rho_{sw}\phi^{-2}$. On the right, divisions between layers 2A, 2B and 2C have been picked arbitrarily, based on changes in the apparent porosities shown here.

recovered cores[3]. Finer scale variations in apparent resistivities (and our later porosity estimates), particularly within the pillow lavas, may be related to the variable degree of alteration[3], either by partial filling of pore spaces, or because of the enhanced conductivities of alteration products. Comparison of our *in situ* resistivities with typical values of 5–3,000 Ω m measured on basalt samples[11–13] suggests a significant fracture porosity in the pillow lavas, sharply decreasing into the dykes below.

Considerably greater detail in apparent resistivities is shown by the much smaller-spaced Schlumberger spherically-focused laterolog (SFL) run on Leg 83, even when these borehole-corrected readings were averaged over 5 m (Fig. 4). As the radius of investigation of the SFL is relatively small (~0.5 m), some of this apparent detail is not directly representative of *in situ* conditions, but instead reflects some combination of exaggerated tool response at resistivity contrasts and the effect of the disturbed zone near the borehole. Nevertheless, average SFL resistivity values agree reasonably well with the large-scale values, except in the deeper, highly resistive section. We attribute this discrepancy to the greater contrast in the deeper section between the resistivity of the disturbed zone and *in situ* resistivity, as well as to possible errors in borehole-correcting the SFL response for the seawater in the hole. We take the large-scale resistivity values to be much better representative of *in situ* resistivity.

Bulk porosity from apparent resistivity

In commercial well logging, the porosity, ϕ, is often obtained from the measured resistivity, ρ_m, and the resistivity of the pore fluids, ρ_f, by application of the empirical Archie's law[14]:

$$\frac{\rho_m}{\rho_f} = a\phi^{-m}$$

with $a \sim 1$, $m \sim 2$. Although this relationship was developed for sedimentary rocks of relatively uniform porosity[15], it has been applied with reasonable success to samples of both oceanic[11–13] and continental[16,17] basement. We cannot rigorously justify application of Archie's law to *in situ* resistivity measurements in the oceanic crust, particularly because of the three-fold nature of the porosity[18]: electrical conduction may be due to vesicular (pore), crack or fracture porosity. The form of the resistivity–porosity relationship depends on the nature of the porosity: exponents near but possibly >2 have applied to measurements on basalt samples with dominantly grain-boundary (microcrack) porosity[11–13,16,17], whereas exponents <2 have applied in interpreting logging measurements in the fractured upper levels of oceanic basement[19,20].

As Hole 504B clearly penetrated through the highly fractured and partially sealed pillow lavas, into relatively unfractured and less altered dykes[3], we cannot realistically expect a single resistivity–porosity relationship to hold throughout. Nevertheless, we roughly approximated bulk porosities by applying Archie's law, compromising on an exponent of 2. The salinity of the pore fluids closely corresponded to that of seawater[21], so we used the temperature-sensitive resistivity of seawater. Based on laboratory measurements[22–24], we applied a linear relationship[9] of seawater conductivity, σ_{sw} (S m^{-1}), with temperature, T(°C),

$$\rho_{sw}^{-1} = \sigma_{sw} = 3 + T/10$$

over the ~160° range of equilibrium crustal temperatures[6].

Figure 5 shows apparent bulk porosities calculated using data from the longest-spaced Leg 83 electrode pair, 3–4, which yielded the largest-scale averages of *in situ* resistivity. Immediately obvious is a striking stratification of porosity, directly corresponding to layers 2A, 2B and 2C, as interpreted from other geophysical measurements in the hole[3]. We obtained bulk porosities of ~12–14% in the highly permeable aquifer, about 100 m thick, identified as a thin layer 2A (ref. 3), 7–10% in the deeper pillows that form layer 2B, and <3% in the dykes and massive units of layer 2C. These values are lower than porosity estimates of 13% or higher obtained from logging layer 2B in Atlantic DSDP holes in older crust[19,20].

A borehole seismic experiment[25] in Hole 504B showed a clear layer 2B/2C break, consistent with our sharp porosity change, but it showed no evidence for layer 2A. However, a ~100-m thick layer 2A may be beyond the resolution of both that experiment and conventional refraction techniques[26]. The Leg 83 sonic logs (Fig. 5 of ref. 3) are not inconsistent with our three-layer stratification of apparent bulk porosity.

Shipboard measurements of grain-boundary porosities of homogeneous basalt samples from Hole 504B yielded mean values of 5±2% in the pillows of layer 2A+2B (refs 12, 13), 3±2% for the entire Leg 83 section, and ~1% in the deeper dykes[27]. Thus our results corroborate interpretations based on other Leg 83 measurements[3,28], namely that: (1) a significant, unsealed fracture porosity exists in layer 2A; (2) fracture porosity is partially sealed by alteration products in layer 2B; and (3) very little fracture porosity exists in the dykes of layer 2C. This low layer 2C fracture porosity may have resulted from sealing by compression or infilling by alteration products. An alternative possibility must be considered—that the dykes may always have been relatively unfractured, which would probably require that hydrothermal circulation never penetrated significantly into layer 2C. In support of this hypothesis, we note that both fracture intensity and degree of alteration, determined *in situ* and from recovered samples, drop significantly in the dykes[3,28].

While we have no firm basis for confidence in our values of bulk porosity, we are confident that the porosity stratification revealed by applying Archie's law is not an artefact of the resistivity–porosity model used. In particular, the hundred-fold increase of resistivity into the dykes strongly implies a sharp reduction in bulk porosity, to 1% or lower. This result is consistent with the interpretation of a sea floor electromagnetic experiment on the flank of the East Pacific Rise at 21 °N, which suggests a similar increase of resistivity at a depth of ~1.4 km (ref. 29). In both that experiment and ours, the remarkable result was the surprisingly shallow level at which apparent resistivity sharply increases, and, by inference, fracture porosity and permeability to hydrothermal circulation decrease significantly.

Conclusions

We have successfully measured *in situ* electrical resistivity throughout nearly 1 km of layer 2 of the oceanic crust at Hole 504B. Crustal resistivities are about 10 Ω m in the pillow lavas forming layers 2A and 2B, and strongly increase to nearly 1,000 Ω m in the dykes and massive units of layer 2C. Bulk porosities estimated from crustal resistivities indicate three porosity layers directly corresponding to layers 2A, 2B and 2C. Our results suggest that there is almost no fracture porosity in layer 2C. The sharp increase with depth in apparent resistivity, and order of magnitude decrease in apparent bulk porosity, are highly consistent with the steep reduction with depth in bulk permeability also observed in the hole[3,7,30]. An important implication of the significant reduction in fracture porosity and permeability with depth, particularly into the dykes, is that any hydrothermal circulation must presently be limited to shallow crustal levels on the flank of the Costa Rica Rift (~1 km), or mostly confined to the pillow basalts. We speculate that hydrothermal circulation may have been limited in this way closer to the spreading axis.

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Construction of influenza haemagglutinin genes that code for intracellular and secreted forms of the protein

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The DNA sequences encoding the amino-terminal signal peptide or the carboxy-terminal hydrophobic anchor have been deleted from a cloned gene coding for the haemagglutinin (HA) of influenza virus. The wild-type gene has previously been shown to be expressed with high efficiency from simian virus 40 (SV40)-HA recombinant vectors into a fully glycosylated protein that is displayed on the infected cell's surface in an antigenically and biologically active form. The anchor-minus HA also is glycosylated but is secreted efficiently into the medium. By contrast, the signal-minus HA is produced only at low levels, is not glycosylated and is located intracellularly.

HYDROPHOBIC amino acid sequences have a crucial role in determining the biosynthetic pathway and ultimate destinations of secretory and integral membrane proteins of eukaryotic cells. The precursors of such proteins invariably contain at or very near their N-termini a tract of hydrophobic amino acids which functions in the translocation of the nascent polypeptide across the lipid bilayer of the endoplasmic reticulum[1-6]. In addition, the majority of membrane proteins have at their C-termini another hydrophobic region that serves to anchor the completed protein into the lipid bilayer[6-10]. Analyses of both types of sequences from a large number of proteins have revealed a remarkable diversity of amino acid sequence, although hydrophobicity is always maintained. These results suggest that the essential difference between a secretory and an integral membrane protein is the presence or absence of an anchoring C-terminal hydrophobic peptide; and they raise the possibility that removal of the signal sequence might divert a secreted protein from its normal biosynthetic pathway into the cytoplasm of the cell.

Influenza virus haemagglutinin (HA) is the best characterized of all integral membrane proteins. Its complete amino acid sequence has been deduced[6,11-16] and its three-dimensional structure[17], organization into trimeric structures[17,18] and orientation with respect to the membrane[19] have been determined. In addition, the location of its major antigenic sites[20] and the points at which the protein is glycosylated[6,17] have been defined. Apart from the specialized features associated with its antigenicity and biological roles of attachment[21] and penetration[22,23], the structure of HA is characteristic of the major class of cellular integral membrane glycoproteins, containing typical terminal hydrophobic regions.

Recently the complete RNA gene for HA from the A/Japan/305/57 strain of influenza virus was converted to double-stranded DNA, cloned, sequenced and inserted into a

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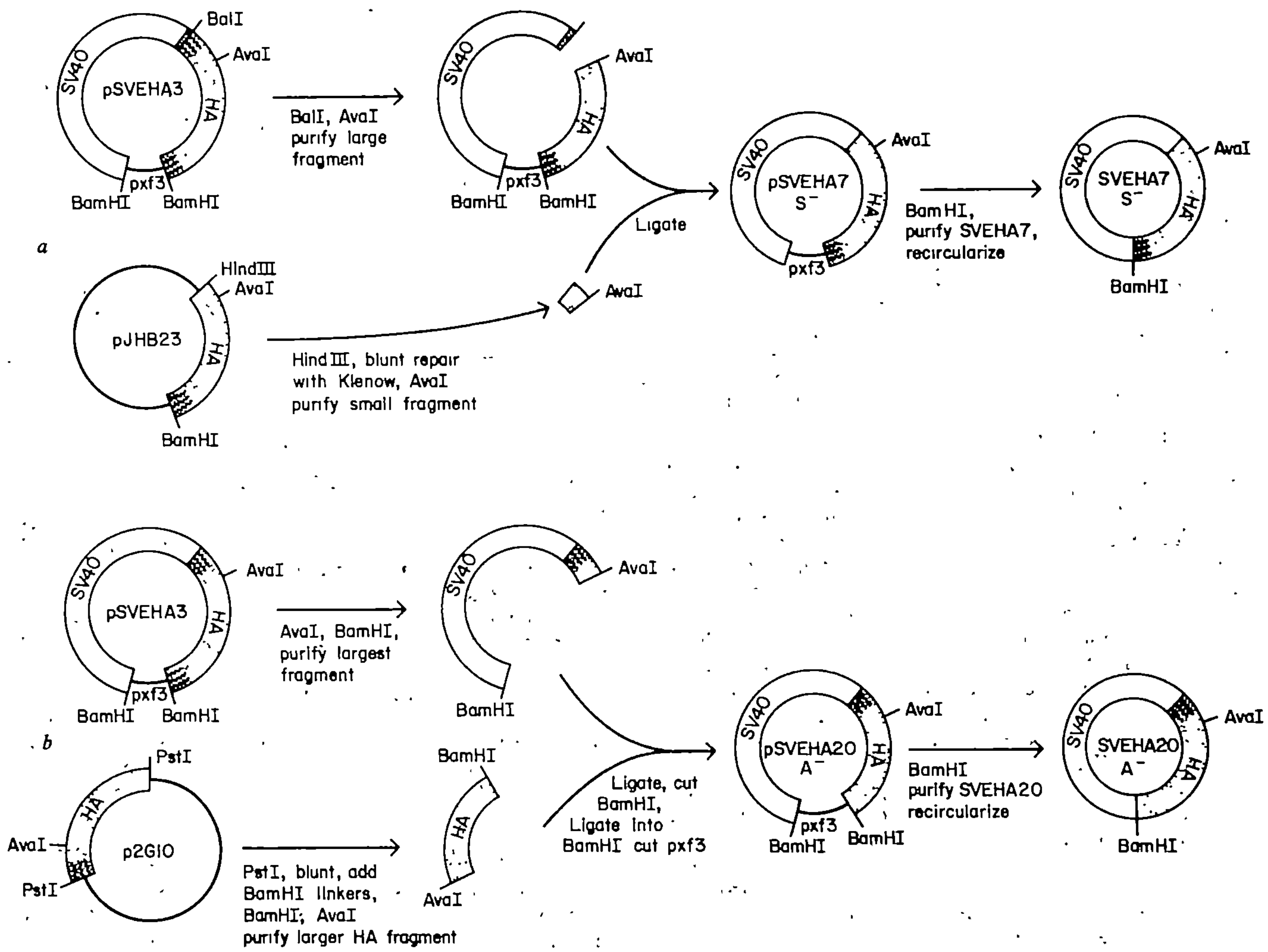

Fig. 1 Construction of recombinant vectors containing mutant genes that lack nucleotide sequences coding for the terminal hydrophobic regions of HA. *a*, The construction of SVEHA7-S⁻ (signal-minus) used two previously synthesized plasmids: pSVEHA3 which consists of the late replacement vector SVEHA3[25] (wild-type HA sequence) cloned into plasmid pXf3 (ref. 28), and pJHB23 which contains a truncated HA gene that was created by *Bal*31 digestion of the wild-type gene[25] and lacks the nucleotides coding for the signal peptide. pSVEHA3 was digested with *Bal*I and *Ava*I and the larger fragment was purified by polyacrylamide gel electrophoresis. (Restriction endonuclease digestions were carried out using the conditions recommended by New England Biolabs. Other cloning procedures were done as described in Maniatis *et al.*[28].) pJHB23 was digested with *Hin*dIII and the protruding tails were filled in using the Klenow fragment of DNA polymerase I. The plasmid was then further digested with *Ava*I and the smallest restriction fragment was purified by polyacrylamide gel electrophoresis. The two fragments were ligated using T4 DNA ligase and transfected into *E. coli* DH-1. A plasmid, pSVEHA7-S⁻, that had the desired structure was identified by restriction endonuclease digestion and the extent of the deletion was verified by determination of the nucleotide sequence across the junction[49]. This plasmid was then digested with *Bam*HI and the SV40-HA fragment was purified by gel electrophoresis and circularized by ligation at low DNA concentration (3 μg ml^{-1}) to produce the recombinant vector SVEHA7-S⁻. *b*, The construction of SVEHA20-A⁻ (anchor-minus) also used pSVEHA3, together with the plasmid p2G10 that lacks the HA nucleotide sequences coding for the C-terminal anchor peptide[6]. pSVEHA3 was digested with *Ava*I and *Bam*HI and the larger fragment was purified. p2G10 was digested with *Pst*I, the protruding tails were removed using S_1 nuclease and *Bam*HI linkers (Collaborative Research) were added. After digestion with *Bam*HI and *Ava*I, the 1,300-nucleotide HA fragment was purified. The two fragments were ligated and transfected into *E. coli* DH-1. A plasmid, SVEHA20-A⁻, that had the desired structure was identified and verified as described above, and the recombinant vector, SVEHA20-A⁻, was produced by purification and ligation.

variety of prokaryotic and eukaryotic expression vectors[6,24–27]. When this version of the HA gene is introduced into eukaryotic cells using vectors derived from SV40, it is expressed as a protein whose structure and biological properties are indistinguishable from those of authentic HA[25]. These cloned, expressing copies of the HA gene provide the material to analyse the effects of mutations on the structure and function of the molecule. Our initial experiments have focused on an analysis of deletion mutants of HA that lack the sequences coding for the N- or C-terminal hydrophobic peptides.

Construction of deletion mutants

The late replacement vector SVEHA3 consists of the coding sequences of the wild-type HA gene inserted into the late region of SV40[25]. This vector was used as the basis for the construction of two new vectors containing mutant HA genes that lack sequences coding for either the N-terminal or C-terminal hydrophobic peptides of the protein. In neither case did the deletions affect the placement of the HA coding sequences with respect to the upstream, controlling SV40 sequences. The construction of these mutants is described in Fig. 1. The signal-minus mutant (SVEHA7-S⁻) was derived from a plasmid (pJHB23) that contains a HA gene from which the 5′ non-translated sequences, the initiation codon and the nucleotides coding for the signal peptide had been removed by digestion with exonuclease *Bal*31 (ref. 25). A restriction fragment containing these truncated 5′ sequences was used to replace the sequences of the wild-type late replacement vector (pSVEHA3) lying between the *Bal*I site immediately following the ATG of the HA gene and the *Ava*I site at nucleotide 340 (ref. 6). This resulted in a mutant HA gene in which the initiation codon was fused in phase to the codon for the first amino acid of the

Table 1 Comparison of the properties of wild-type and mutant HAs produced from SV40-HA vectors

		SVEHA3 Wild-type	SVEHA20-A$^-$ Anchor-minus	SVEHA7-S$^-$ Signal-minus
Location		Cell surface	Secreted to medium	Intracellular
M_r (subunit)		75,000	72,000	63,000
Structure		Trimer	Trimer	?
Glycosylation		+	+	–
Red cell binding to infected cells		+	–	–
HA activity in cell extracts or medium		+	–	–
Cell–cell fusion (low *p*H dependent)		+	–	–
Molecules per cell (60 h post-infection)		6×10^8	1×10^9	1×10^6
Amount produced per 10 cm dish	(Extract)	200 μg	28 μg	0.5 μg
(60 h post-infection)	(Medium)	0	300 μg	0

The subunit structure of the purified proteins was determined by cross-linking with dimethyl suberimidate[18]. Red cell binding was assayed as described previously[25]. Cell–cell fusion was measured by the method previously described[23].

mature HA1 polypeptide (Fig. 2). The remainder of the HA coding sequences were unaltered. The anchor-minus mutant (SVEHA20-A$^-$) was derived from a plasmid (p2G10) containing a copy of the HA gene that lacks sequences coding for the C-terminal hydrophobic peptide and the putative cytoplasmic tail of the protein[6]. An *AvaI–BamHI* restriction fragment from this truncated gene was used to replace the corresponding wild-type HA sequence in pSVEHA3 (Fig. 1).

The mutant forms of the late replacement vectors were cloned in plasmid pXf3 (ref. 28) and propagated in *Escherichia coli.* The novel nucleotide sequences formed as a consequence of the deletions were determined and the amino acids encoded by the altered regions are shown in Fig. 2. The HA protein encoded by SVEHA7-S$^-$ lacks 14 amino acids that comprise the wild-type signal peptide. These sequences are replaced by a tract of three amino acids—Met, Glu, Leu—that is clearly neither long enough nor sufficiently hydrophobic to function as a signal peptide. On the other hand, the protein that is encoded by SVEHA20-A$^-$ lacks 38 amino acids from the C-terminus of the wild-type HA, including the hydrophobic anchoring sequence and the short hydrophilic tail. These residues are replaced in the mutant by 11 predominantly charged amino acids encoded by nucleotides from the GC homopolymer tails, the *BamHI* linker and a short stretch of SV40 sequence which precedes the first in-frame termination codon.

When the sequences of the mutants had been verified, the SVEHA recombinant genomes were excised from their plasmids by digestion with *BamHI*, purified by gel electrophoresis and recircularized by ligation at low DNA concentration[25]. The resulting circular DNA molecules were then introduced together with a helper virus genome, dl1055 (ref. 29), into CV-1 cells using DEAE–dextran as the facilitator[30]. Virus stocks were prepared as described previously[25].

```
                                                                  signal cleavage
                                                                        ↓
SIGNAL+   NH2 H Met.Ala.Ile.Ile.Tyr.Leu.Ile.Leu.Leu.Phe.Thr.Ala Val Arg.GLY.ASP GLN ILE CYS ILE - HA1
                -15                -10                -5              -1   1               5
SIGNAL-                                                  NH2 - Met.Glu Leu.GLY.ASP GLN.ILE.CYS.ILE - HA1

                                       ***
ANCHOR+   HA2 - LYS LEU.SER.SER.MET.GLY.VAL.TYR.Gln Ile.Leu Ala Ile.Tyr.Ala Thr -
                502
ANCHOR-   HA2 - LYS.LEU SER.SER.MET.GLY.VAL TYR Pro Pro.Pro.Gly Ser.Arg.His Asp.-
                502

ANCHOR+       -.Val.Ala.Gly.Ser.Leu Ser.Leu.Ala.Ile.Met.Met.Ala.Gly.Ile.Ser.Phe.-

ANCHOR-       -.Lys.Ile.His - COOH
                        520
                        ***
ANCHOR+       -.Trp.Met.Cys Ser.Asn.Gly.Ser Leu.Gln.Cys.Arg.Ile.Cys.Ile - COOH
                                                            547
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Fig. 2 Comparison of the amino acid sequences coded by wild-type and mutant HA genes. The novel nucleotide sequences in the mutant HA genes, formed as a consequence of the deletions, were determined by the Maxam–Gilbert technique[49]. The amino acid sequences in the altered regions are compared with the wild-type sequence. Residues that are conserved between mutant and wild-type proteins are shown in capital letters, while those that differ are indicated by lower case letters. *** Indicates the probable beginning and end of the C-terminal hydrophobic sequence that is inserted in the lipid bilayer.

Expression of variant forms of HA from SV40-HA recombinants

Monolayers of CV-1 cells were infected at high multiplicity with vectors carrying the wild-type and mutant forms of the HA gene. Sixty hours later the media above the monolayers were collected and cell extracts were prepared[25]. The amount of HA antigen in aliquots of the supernatants and cell extracts was measured by a solid phase radioimmunoassay[25] and the results are shown in Table 1. HA expressed from the SVEHA3 vector is found exclusively in a cell-associated form: previous work[25] has shown that it is anchored into the plasma membrane. By contrast, the anchor-minus HA expressed from SVEHA20-A$^-$ is found in the medium in very high amounts, indicating that it is secreted from infected cells. The small fraction of HA antigen found in the cell extract may represent nascent protein that is still being transported within the cell or secreted material that has adsorbed to the cell surface. Both the wild-type and the anchor-minus HA are very efficiently expressed from the recombinant vectors, with up to 10^9 molecules of HA synthesized in each infected cell. Once the infection is well established (~20 h post-infection) serum-containing medium is no longer necessary for the continued production and secretion of HA. Thus, at 60 h after infection, 40 h after changing to serum-free medium, some 300 μg of essentially pure HA can be collected from a single 10-cm plate of cells infected with the anchor-minus mutant. Much less HA antigen is synthesized in cells infected with SVEHA7-S$^-$. Only 0.5 μg of HA is produced per dish, all of which is cell-associated.

Characterization of HA mutants

Supernatants and extracts obtained from cells radiolabelled with ^{35}S-methionine between 48 and 49 h after infection were immunoprecipitated and analysed by SDS-polyacrylamide gel electrophoresis (PAGE). Figure 3 shows the effects of the mutations on the size and location of the HA protein. As described previously[25], cells infected with SVEHA3 express a protein of molecular weight (M_r) 75,000 that is specifically precipitated by anti-HA serum from cell extracts but not from the supernatant medium. However, the HA protein synthesized during the 1-h labelling period by cells infected with SVEHA20-A$^-$ can be precipitated from both the cell extract and the medium, thus confirming that it is secreted from the cells. Both the wild-type and secreted forms of HA can be seen as major bands when the proteins in the labelled extracts are separated by SDS-PAGE without previous immunoprecipitation (Fig. 3). The slightly lower molecular weight of the secreted form of HA is as expected because the mutant gene encodes a protein that is 27 amino acids shorter than the wild-type molecule (Fig. 2).

A smaller protein, produced in much reduced quantities, is precipitated from extracts of cells infected with SVEHA7-S$^-$. In size, this protein is the same (M_r 61,000) as the non-glycosylated HA synthesized *in vitro* by translation of wild-type HA

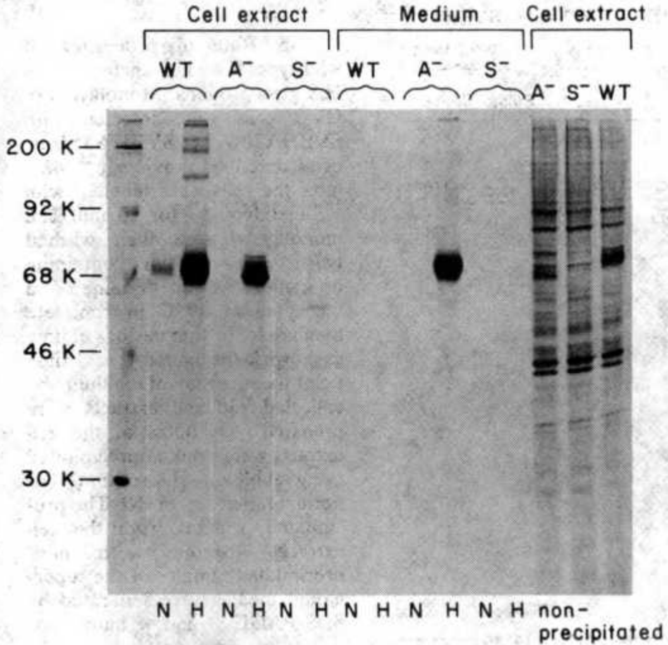


Fig. 3 Comparison of the expression of wild-type and mutant HA genes. Monolayers of CV-1 cells were infected with SVEHA3 (WT), SVEHA7-S⁻ (S⁻) or SVEHA20-A⁻ (A⁻) viruses as described previously[25]. 48 h later the cells were labelled for 1 h with ^{35}S-methionine and then the media above the cells were collected and cell extracts were prepared[25]. Aliquots of these samples were immunoprecipitated using rabbit anti-HA serum (H) or normal rabbit serum (N). The precipitated proteins were separated by SDS-PAGE and autoradiographed[25].

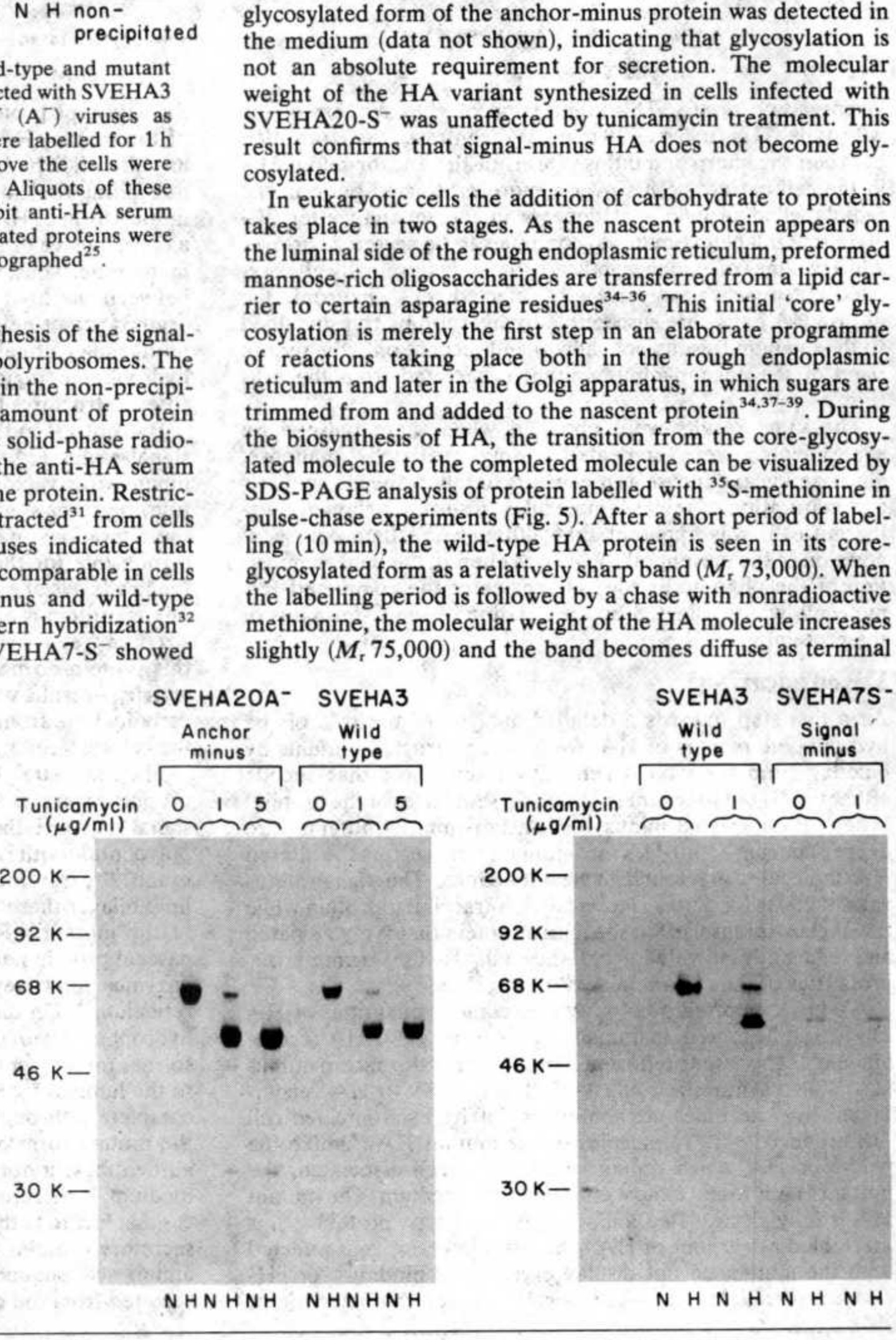
mRNA[25]. This result is consistent with synthesis of the signal-minus HA as a cytoplasmic protein on free polyribosomes. The failure to detect a major band of M_r 61,000 in the non-precipitated sample indicates that the decreased amount of protein observed both in this experiment and in the solid-phase radioimmunoassay is not due to the inability of the anti-HA serum to recognize the non-glycosylated form of the protein. Restriction endonuclease analysis of viral DNA extracted[31] from cells infected with the various recombinant viruses indicated that the numbers of recombinant genomes were comparable in cells infected with the anchor-minus, signal-minus and wild-type SV40-HA recombinants. However, Northern hybridization[32] of RNA purified from cells infected with SVEHA7-S⁻ showed that the level of HA-specific sequences was several-fold lower than in cells infected with the other recombinants. Furthermore, experiments in which infected cells were labelled for short times with ^{35}S-methionine and then chased showed that the signal-minus HA was more rapidly degraded than the wild-type protein. Taken together, these results indicate that the low yield of signal-minus HA may be due to instability of both the mutant mRNA and its protein product.

Analysis of glycosylation of HA mutants

To ascertain whether the various HA species were glycosylated, infected cells were labelled with ^{35}S-methionine in the presence of tunicamycin, a drug that blocks the formation of the dolichol-oligosaccharide donor and thus prevents primary glycosylation of the nascent polypeptide[33]. SDS-PAGE analysis of the resulting proteins is shown in Fig. 4. Tunicamycin treatment of cells infected with SVEHA3 or SVEHA20-A⁻ resulted in a reduction in the sizes of the immunoprecipitated proteins to M_rs of 61,000 and 58,000, respectively. These sizes are the same as those of the non-glycosylated forms of the protein synthesized *in vitro* by translation of wild-type and anchor-minus HA mRNA (data not shown). A small proportion of the non-glycosylated form of the anchor-minus protein was detected in the medium (data not shown), indicating that glycosylation is not an absolute requirement for secretion. The molecular weight of the HA variant synthesized in cells infected with SVEHA20-S⁻ was unaffected by tunicamycin treatment. This result confirms that signal-minus HA does not become glycosylated.

In eukaryotic cells the addition of carbohydrate to proteins takes place in two stages. As the nascent protein appears on the luminal side of the rough endoplasmic reticulum, preformed mannose-rich oligosaccharides are transferred from a lipid carrier to certain asparagine residues[34-36]. This initial 'core' glycosylation is merely the first step in an elaborate programme of reactions taking place both in the rough endoplasmic reticulum and later in the Golgi apparatus, in which sugars are trimmed from and added to the nascent protein[34,37-39]. During the biosynthesis of HA, the transition from the core-glycosylated molecule to the completed molecule can be visualized by SDS-PAGE analysis of protein labelled with ^{35}S-methionine in pulse-chase experiments (Fig. 5). After a short period of labelling (10 min), the wild-type HA protein is seen in its core-glycosylated form as a relatively sharp band (M_r 73,000). When the labelling period is followed by a chase with nonradioactive methionine, the molecular weight of the HA molecule increases slightly (M_r 75,000) and the band becomes diffuse as terminal

Fig. 4 Effect of tunicamycin on the HA proteins expressed from wild-type and mutant genes. Monolayers of CV-1 cells, infected with the recombinant vectors as described previously[25], were treated with tunicamycin (0, 1 or 5 μg ml⁻¹ in DME containing 10% serum) between 46 and 48 h after infection. The cells were then labelled with ^{35}S-methionine in the presence of the same concentrations of tunicamycin. Cell extracts were prepared, immunoprecipitated, separated by SDS-PAGE and autoradiographed[25].



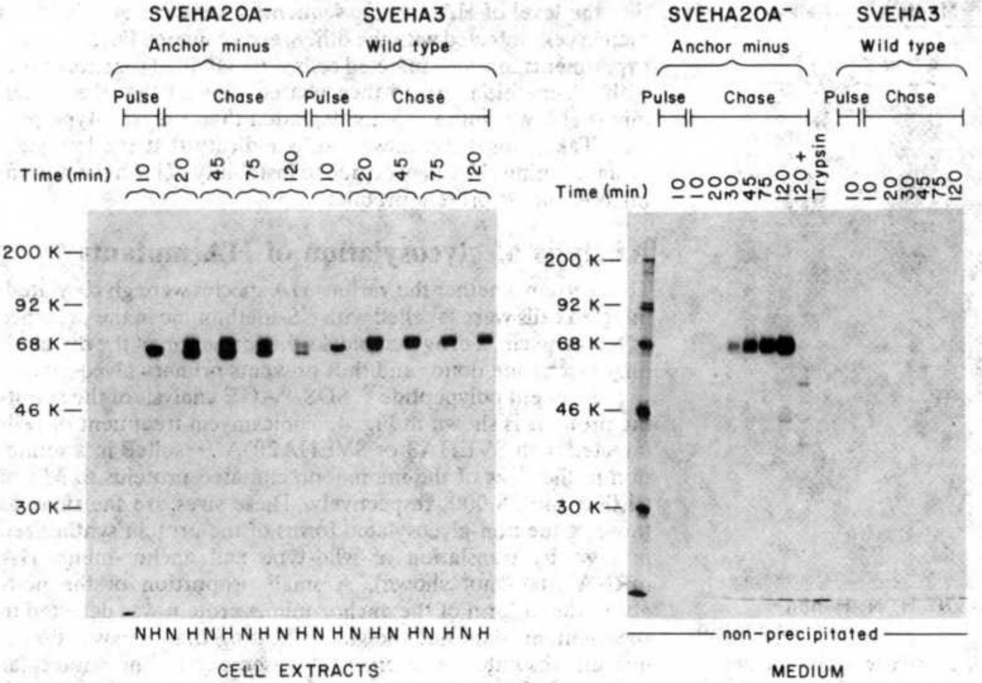


Fig. 5 Rate of processing of wild-type and anchor-minus HA glycoproteins. Monolayers of CV-1 cells were infected with SVEHA20-A⁻ or SVEHA3 viruses as described previously[25]. 48 h later the cells were labelled with ^{35}S-methionine[25] for 10 min. The monolayers were then washed twice with medium containing nonradioactive methionine and incubated at 37 °C in complete medium for further periods of time as shown in the figure. At each time point the supernatant medium was collected and cell extracts were prepared[25]. Aliquots of the cell extracts were immunoprecipitated using rabbit anti-HA serum (H) or normal rabbit serum (N). The precipitated proteins from the cell extracts, together with non-precipitated samples of the supernatant media, were separated by SDS-PAGE and autoradiographed[25].

glycosylation occurs. This completion of glycosylation of the wild-type HA takes ~20 min. By contrast, the transition between the sharp and diffuse forms of the anchor-minus HA in the cell extract occurs over a more protracted period. The radiolabelled protein first appears in the medium after 20–30 min and labelled material continues to be secreted for over 2 h. Two lines of evidence indicate that only terminally glycosylated HA reaches the surface of infected cells: first, only the diffuse HA bands are susceptible to digestion by trypsin added to the medium (results not shown) and second, only the diffuse band of the anchor-minus mutant is secreted from the cells (Fig. 5).

The same results were obtained when these pulse-chase experiments were repeated using tritiated mannose, glucosamine and fucose as precursors to follow the biosynthesis and maturation of the HA molecules (results not shown). Furthermore, it was apparent that although the time course of addition of the peripheral sugars was altered, the final composition of the oligosaccharide side chains of the completed HA molecule was unaffected by the deletion of the C-terminus of the protein.

Discussion

As a first step towards a detailed analysis of the role of the hydrophobic regions of HA, we have constructed mutants by deleting from the cloned gene DNA sequences that encode either the N- or C-terminal amino acid sequences of the protein. When these signal-minus or anchor-minus mutants are expressed from SV40-HA recombinant viral vectors the altered HA molecules are found in new locations. The signal-minus variant codes for a non-glycosylated, intracellular protein while the anchor-minus variant specifies a protein that is glycosylated and efficiently secreted from the cell. Table 1 summarizes properties of the mutant and wild-type HAs.

We have reported previously that copious quantities of HA can be synthesized in mammalian cells from SV40-HA recombinants[25]. The results reported here show that the anchor-minus HA is also synthesized efficiently from an SV40-HA recombinant. By late times after infection (60 h), each infected cell has produced $\sim 10^9$ molecules of the mutant HA. Unlike the wild-type HA which remains exclusively cell-associated, the mutant HA is found almost entirely in the medium. The mutant HA is fully glycosylated and, like the wild-type protein[17,18], is assembled as a trimer of HA subunits. However, cells infected with the mutant do not display erythrocyte binding[25] or *p*H-dependent cell fusion[23]—biological activities characteristic of the wild-type protein. From the predicted amino acid sequence of the mutant HA there would be no reason to suppose that any alteration had occurred in the structure or function of either its binding site[17] or of its fusion peptide[17,40]. The failure of the mutant form of HA to display these functions is almost certainly a consequence of its never being an integral part of the plasma membrane. Thus, the protein cannot form the necessary bridge between the lipid bilayer of the infected cell and that of the erythrocyte or neighbouring cell. Finally, the secreted HA has no haemagglutinating activity because, lacking the C-terminal hydrophobic region, the protein cannot aggregate to form rosette structures that bind erythrocytes polyvalently.

By contrast to the wild-type and anchor-minus proteins, the signal-minus HA is expressed at low levels from the recombinant virus vector (10^6 molecules per cell at 60 h post-infection). Further studies are underway to determine in what way the alterations in sequence at the 5′ end of the gene may be responsible for the instability of the mutant mRNA. Despite the low levels of expression, our results indicate that the signal-minus protein is an intracellular form of HA probably synthesized on free polyribosomes in the cytoplasm. Cells expressing this protein do not display erythrocyte binding or cell fusion activity—results which are consistent both with the absence of carbohydrate from the molecule[41] and with its failure to reach the cell surface.

The most straightforward explanations of the two mutants' properties are as follows. In the absence of the N-terminal signal sequence there can be no interaction of the nascent HA polypeptide with receptors on the endoplasmic reticulum membrane[42,43]; translocation of the growing polypeptide across the lipid bilayer therefore cannot occur. Consequently, translation of the mutant mRNA takes place in the cytoplasm and the nascent protein never comes into contact with the glycosylating enzymes that reside in the lumen of the endoplasmic reticulum[34]. On the other hand, the removal of the C-terminal hydrophobic sequence results in a loss of anchoring function, so that the nascent polypeptide, instead of remaining attached to the luminal face of the rough endoplasmic reticulum, passes completely through the membrane. Once free in the lumen, the mutant form of HA is treated by the cell as if it were an authentic secretory protein, and it is discharged into the medium. It therefore follows that apart from the signal sequences that facilitate their transport into the endoplasmic reticulum, secretory proteins as a class do not contain additional specific amino acid sequences that confer on them the ability to be secreted from the cell.

It is generally assumed that all secretory and integral membrane proteins are transported in vesicles from their site of synthesis on the endoplasmic reticulum via the Golgi apparatus to the plasma membrane. However, it is not known whether the two types of protein are sequestered into different vesicles or whether they follow exactly the same intracellular route. Although the number of proteins studied remains small, it appears that in general secreted proteins move more slowly than membrane proteins to the cell surface and that most, if not all, of this difference in rate arises in the part of the intracellular pathway between the endoplasmic reticulum and the Golgi apparatus[44]. Our results indicate that the transport of the two forms of HA also conforms to this pattern. Thus, by contrast to the membrane-bound wild-type protein which is glycosylated rapidly and relatively synchronously, the population of secretory molecules becomes terminally glycosylated over a very protracted period. It is possible that this difference in some measure reflects the relative efficiency with which membrane-bound proteins and luminal proteins are sequestered into the transport vesicles that travel from the rough endoplasmic reticulum to the Golgi apparatus. Even though the rate of glycosylation of anchor-minus HA is comparatively slow, the composition of the oligosaccharides attached to the wild-type and anchor-minus forms of the protein are not significantly different. It therefore appears that both the secreted and membrane-bound forms of the protein undergo the same sequence of modification reactions during their passage through the Golgi apparatus, and that the signals governing the pattern of glycosylation of HA cannot reside in the 38 amino acids that normally comprise the C-terminus of the protein.

While the mutant forms of HA have been artificially created *in vitro* by deleting portions of the gene, natural counterparts of these variants are found in eukaryotic cells. *Saccharomyces cerevisiae* produces, from a single gene, distinct mRNA species that code for secreted and cytoplasmic forms of invertase[45]. The secreted enzyme, which is glycosylated, is derived from a precursor that carries a 19-residue N-terminal signal sequence. By contrast, the cytoplasmic form of the enzyme is neither glycosylated nor derived from a precursor. In murine lymphocytes a single gene codes for both membrane-bound and secreted forms of immunoglobulin heavy chains[10,46,47]. Developmentally regulated alternative pathways of RNA processing give rise to either of two mRNAs, one of which encodes the secreted protein with a hydrophilic C-terminus, while the second encodes in addition a hydrophobic extension that anchors the protein in the membrane.

Finally, the studies with HA can be extended in several ways. For example, the functions of the hydrophobic regions can be analysed in fine detail by creating smaller deletions, insertions and point mutations at particular sites within the cloned HA gene. It will also be possible to construct genes coding for chimaeric proteins, in which the various functional domains of one membrane protein are exchanged for those of another. In this way it can be determined if signal and anchor sequences can be interchanged between different membrane proteins. Meanwhile, the work already accomplished is an illustration of what may become a general method for obtaining with ease large quantities of purified eukaryotic membrane antigens for experimental analysis or vaccine production. (After this manuscript was submitted a paper was published by Sveda *et al.*[48] on a similar topic.)

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Asymmetric ligand binding by haemoglobin

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The asymmetry observed in the titration curve of haemoglobin with oxygen is possible only if the α-α and β-β subunit interactions change by different amounts on oxygenation. This necessary difference justifies the $\alpha_2\beta_2$ structure on functional grounds and its magnitude shows that haemoglobin function results from reversible oxygen binding to the β subunit.

THE binding of oxygen to haemoglobin is generally presented as a plot of the logarithm of the oxygen concentration against the number of ligands bound per protein molecule (Bjerrum plot). Allen, Guthe and Wyman[1] obtained as the condition of symmetry of this plot about the midpoint a relation between the four dissociation constants: $K_1K_4 = K_2K_3$, and observed that it required symmetric haem–haem interactions. They believed that departures from symmetry were probably

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Table 1 Relative oxygen dissociation constants, asymmetries and computed binding constraints (kcal mol^{-1}) of haemoglobin A

Condition	K_1	K_2	K_3	K_4	Λ	$\{\alpha_o\beta_j\}$	$\{\alpha_o\beta_i\}$	$\{\beta\beta\}$
Stripped[6]	55.2	14.7	5.82	1	+0.44	1.21	0.96	0.57
HB-ATP[6]	345	124	73	1	+3.26	2.00	0.68	2.01
Hb-DPG[6]	545	118	218	1	+3.85	3.24	0.38	2.32
Hb-IHP[5]	127	56	224	1	+4.59	3.24	−0.41	2.72
Hb in 0.1 M phosphate[7]	542	106	153	1	+3.40	3.05	0.55	2.09

Computation of constraints made under the assumptions: $\delta I = 0$; $\{\alpha\alpha\} = 0$, first order constraints (equation (14)). The later work of Tyuma *et al.*[3] agrees well with the data: stripped Hb, $\Lambda = 0.87 \pm 0.35$; DPG-Hb, $\Lambda = 4.99 \pm 0.45$; IHP-Hb, $\Lambda = 4.14 \pm 0.45$.

artificial, but Roughton *et al.*[2] showed that there was significant asymmetry in the titration curve and more recently Tyuma *et al.*[3] saw a very marked increase in asymmetry on addition of phosphoric effectors to stripped haemoglobin. It is the purpose of this article to relate the asymmetry of titration curves for any number of binding sites to the asymmetry in the functions describing the ligand distribution among the protein molecules, and to show that in haemoglobin these asymmetries necessarily require a considerable difference in the change in interaction energies in the α-α and β-β boundaries upon oxygenation.

Symmetry of ligand distribution and of titration curve

When a protein binds several ligands at sites of identical affinity the distribution of ligands among the protein molecules is the normal Bernoulli distribution[4]. If on average $\bar{n}$ ligands are bound per protein molecule at N possible sites, the fraction of protein molecules binding J ligands is

$$f_J(\bar{n}) = \binom{N}{J}(\bar{n}/N)^J(1-(\bar{n}/N))^{N-J} \qquad (1)$$

where $\bar{n}/N$ is the average probability of occupancy of a site. The plots of f_J against $\bar{n}$, which specify the ligand distribution as $\bar{n}$ increases from 0 to N are, in the case of the normal distribution, symmetric about $\bar{n} = N/2$ in the sense indicated by the equation

$$f_J(\bar{n}) = f_{N-J}(N-\bar{n}) \qquad (2)$$

The symmetry of the distributions that are obtained when the binding sites have arbitrary, rather than equal affinities for the ligand may be readily derived. The dependence of $\bar{n}$ upon the concentration of free ligand [X], in equilibrium with the bound species[4,5] is described by an equation of the form

$$\bar{n} = \sum_{J=0}^{N} J\phi_J([\mathrm{X}]) \Big/ \sum_{J=0}^{N} \phi_J([\mathrm{X}]) \qquad (3)$$

$$\phi_J([\mathrm{X}]) = \binom{N}{J} \frac{[\mathrm{X}]^J}{K_1K_2\ldots K_J} \qquad (4)$$

$K_J (1 \leq J \leq N)$ is the intrinsic dissociation constant of the PX_J complex into the components X and PX_{J-1}. The dependence of the distribution of ligands upon [X] is given by

$$f_J(\bar{n}) = \phi_J([\mathrm{X}]) \Big/ \sum_{J=0}^{N} \phi_J([\mathrm{X}]) \qquad (5)$$

Let

$$\sum_{J=0}^{N} \phi_J([\mathrm{X}_{\bar{n}}]) \equiv P_{\bar{n}}; \quad K_1 \ldots K_J \equiv a_J \qquad (6)$$

where $[\mathrm{X}_{\bar{n}}]$ corresponds to an equilibrium with $\bar{n}$ bound ligands. Then equation (2) gives

$$\frac{[\mathrm{X}_{\bar{n}}]^J}{[\mathrm{X}_{N-\bar{n}}]^{N-J}} \frac{a_{N-J}}{a_J} = \frac{P_{\bar{n}}}{P_{N-\bar{n}}} = \frac{[\mathrm{X}_{\bar{n}}]^{J+1}}{[\mathrm{X}_{N-\bar{n}}]^{N-J-1}} \frac{a_{N-J-1}}{a_{J+1}} \qquad (7)$$

which reduces to

$$[\mathrm{X}_{\bar{n}}][\mathrm{X}_{N-\bar{n}}] = K_{N-J}K_{J+1} \qquad (8)$$

Equation (8) shows that a plot of log [X] against $\bar{n}$ symmetric about $N/2$ implies a ligand distribution which is also symmetric about $N/2$. Explicit knowledge of the dissociation constants is unnecessary to demonstrate the symmetry of ligand distribution, the constancy of the product $[\mathrm{X}_{\bar{n}}][\mathrm{X}_{N-\bar{n}}]$ being alone required. Further, equation (8) implies that if the ligand distribution is symmetric about $N/2$, products of pairs of the dissociation constants are equal. The ligand distribution for $N = 2$ is always symmetric. For $N = 3$ equation (9) gives

$$K_3K_1 = K_2^2 \qquad (9)$$

and for $N = 4$

$$K_2K_3 = K_1K_4 \qquad (10)$$

Figure 1 shows plots of log $[\mathrm{X}_{\bar{n}}][\mathrm{X}_{4-\bar{n}}]$ against $\bar{n}$ for two systems, with $N = 4$ and $K_4/K_1 = 43$. K_3K_2/K_1K_4 has been made equal to 4.59 and 1/4.59. Starting from the same point at $\bar{n} = 0$ the plots diverge towards values respectively larger and smaller than the initial one and tend to a constant value as $\bar{n}$ approaches 2. The asymmetry is seen to be of opposite nature according to whether K_4K_1 is smaller or larger than K_2K_3. To indicate the character and magnitude of the binding asymmetry I shall use the quantity

$$\Lambda = \ln(K_2K_3/K_1K_4) \qquad (11)$$

The choice of the logarithm ensures that reciprocal values of the ratio of products of constants are represented by the same figure with opposite sign and reserves $\Lambda = 0$ for the symmetric case. As shown in the figure the logarithmic increment

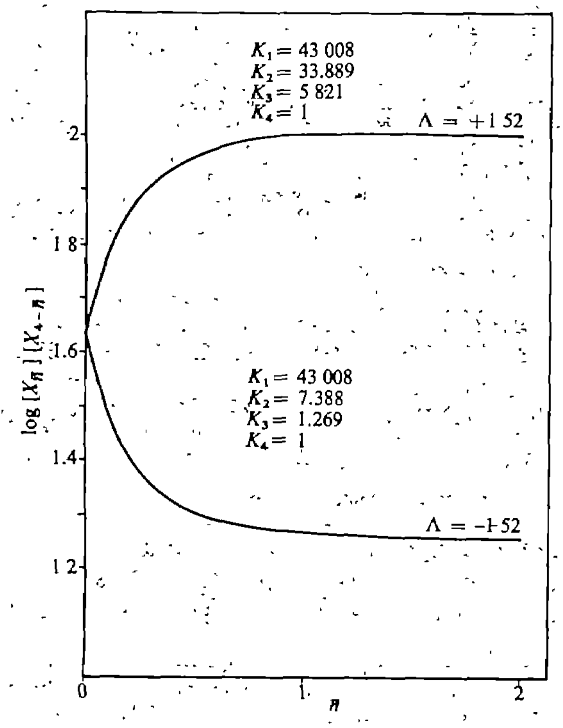

Fig. 1 A plot to demonstrate the opposite effects of negative and positive asymmetry of binding upon the titration curve.

for the positive asymmetry is the same as the logarithmic decrement for the negative one. The significance of positive versus negative asymmetry is disclosed in Fig. 2 which shows plots of f_J against $\bar{n}$ for the systems of Fig. 1. It is a general property of the distributions specified by equation (3) that f_J reaches a maximum at $\bar{n} = J$, whether the distribution is symmetric or otherwise. It is apparent from the figure that in the positive asymmetry the distribution is skewed so that $f_1(1) > f_3(3)$, while for the negative asymmetry the opposite obtains: $f_1(1) < f_3(3)$. Both distributions shown on Fig. 2 have equal Hill coefficients at $\bar{n} = 2$ of ~ 2.4. The result of the skewing towards f_1 in positive asymmetry is that the cooperative character of the binding is retained at values of $\bar{n}$ closer to 4 in comparison with the negative asymmetry, which exhibits the larger cooperativity at $\bar{n} < 1$. Consequently a smaller decrease in ligand concentration is required to produce the same unloading of X from the protein with positive asymmetry, at values of $\bar{n}$ close to 4. This is clearly shown in the Bjerrum plots of Fig. 3. On this basis alone one expects haemoglobin, which physiologically operates within the saturation range of 0.75 and 0.99+ to exhibit positive asymmetry. If the function of haemoglobin was to scavenge oxygen rather than to work as an almost saturated carrier a negative binding asymmetry would be beneficial to its function.

Origin of the asymmetric ligation

"When effects are asymmetric, the asymmetry should be apparent in their causes", said Pierre Curie in 1893[6]. To explain the origin of the asymmetry we take as an example a tetramer of the type $\alpha_2\beta_2$. This will permit direct application to haemoglobin, the only protein aggregate for which structure and properties are known in sufficient detail to permit this kind of analysis. In an $\alpha_2\beta_2$ tetramer there are six possible subunit interactions: of these the interactions at $\alpha_1\beta_1$ and $\alpha_2\beta_2$ and those at $\alpha_1\beta_2$ and $\alpha_2\beta_1$ respectively equal because of the structural symmetry, so that there are only four different interactions that we shall call $\alpha_i\beta_i$, $\alpha_i\beta_j$, $\alpha\alpha$ and $\beta\beta$. To lighten the notation we designate as $\bar{\alpha}$ and $\bar{\beta}$ the Gibbs free energy changes on binding of dioxygen to the subunits, in the absence of changes in subunit interactions, and we designate as $\{\alpha_i\beta_i\}$, $\{\alpha_i\beta_j\}$, $\{\alpha\alpha\}$, $\{\beta\beta\}$ the difference in free energy of the intersubunit binding between unliganded and fully liganded haemoglobin. These differences have the opposite sign to the free energies of binding, if they are binding constraints, and a wealth of observations indicate that in haemoglobin the subunit interactions operate as binding constraints. To compute the effects that may be expected upon ligation it is necessary to specify how the constraints disappear as successive units are liganded and to this purpose we distinguish two possibilities: a first order constraint, which disappears when a subunit at either side of the boundary is liganded, and a second order constraint, which disappears only when the two subunits across the boundary are liganded. Constraints of the third or fourth order may indeed exist but they must have a very secondary importance in relation of those of first or second order and we neglect them in our treatment. The insignificance of constraints of order intermediate between the first and the second will be made clear shortly.

Computation of the binding asymmetry

The Gibbs free-energy levels $G_0, G_1, \ldots, G_4$, corresponding to the species P, PX, ..., PX_4 respectively, are related to the dissociation constants appearing in equation (4) by the equation:

$$\ln K_j = \frac{1}{RT}(G_j - G_{j-1}) \qquad 1 \leqslant j \leqslant 4 \tag{12}$$

If the free-energies are expressed in RT units, as we shall do in the following, RT disappears in the last and all subsequent equations involving the G values and the binding asymmetry expressed as a function of them is, from (11) and (12)

$$\Lambda = G_0 - 2G_1 + 2G_3 - G_4 \tag{13}$$

which shows that computation of Λ does not require the explicit value of G_2. The contribution to the chemical potentials resulting from the interaction of two binding sites is always expressible as a free-energy coupling between them[7]. Binding constraints are antagonistic free energy couplings between the binding of one subunit by another and the binding of either to the ligand. The constraints present when possible sets of sites are liganded are recorded below, where $S = \{\alpha_i\beta_i\} + \{\alpha_i\beta_j\}$

Ligands bound	C (sites filled)	1st order value	2nd order value	
0	C_0	$2S+\{\alpha\alpha\}+\{\beta\beta\}$	$2S+\{\alpha\alpha\}+\{\beta\beta\}$	
1(α)	C_α	$S+\{\beta\beta\}$	C_0	
1(β)	C_β	$S+\{\alpha\alpha\}$	C_0	
2($\alpha\alpha$)	$C_{\alpha\alpha}$	$\{\beta\beta\}$	C_0-$\{\alpha\alpha\}$	
2{$\beta\beta$}	$C_{\beta\beta}$	$\{\alpha\alpha\}$	C_0-$\{\beta\beta\}$	
2($\alpha_i\beta_i$)	$C_{\alpha_i\beta_i}$	$\{\alpha_i\beta_i\}$	C_0-$\{\alpha_i\beta_i\}$	(14)
2($\alpha_i\beta_j$)	$C_{\alpha_i\beta_j}$	$\{\alpha_i\beta_j\}$	C_0-$\{\alpha_i\beta_j\}$	
3($\alpha\alpha\beta$)	$C_{\alpha\alpha\beta}$	0	$S+\{\beta\beta\}$	
3($\alpha\beta\beta$)	$C_{\alpha\beta\beta}$	0	$S+\{\alpha\alpha\}$	
4	C_4	0	0	

The contribution to the Gibbs levels from each ligand configuration of the table amounts to

$$G(\alpha, \beta) = C(\alpha, \beta) + k\bar{\alpha} + m\bar{\beta} \tag{15}$$

where $0 \leqslant k, m \leqslant 2$; $k + m = j$.

Some of the Gibbs levels are manifolds and the contributions of equation (15) must be weighted[4] to obtain the average value. For G_1 and G_3 the weights are:

$$f_\alpha = \exp(C_\alpha + \bar{\alpha})/(\exp(C_\alpha + \bar{\alpha}) + \exp(C_\beta + \bar{\beta})) = 1 - f_\beta \tag{16}$$

$$f_{\alpha\alpha\beta} = \exp(C_\alpha + 2\bar{\alpha} + \bar{\beta})/(\exp(C_\alpha + 2\bar{\alpha} + \bar{\beta}) + \exp(C_\beta + \bar{\alpha} + 2\bar{\beta})) = 1 - f_{\alpha\beta\beta} \tag{17}$$

With the constraints of (14), (13) gives, for first order constraints

$$\Lambda_1 = 2(\bar{\alpha} - \bar{\beta})[f_{\alpha\alpha\beta} - f_\alpha] - [\{\alpha\alpha\} - \{\beta\beta\}](2f_\alpha - 1)$$

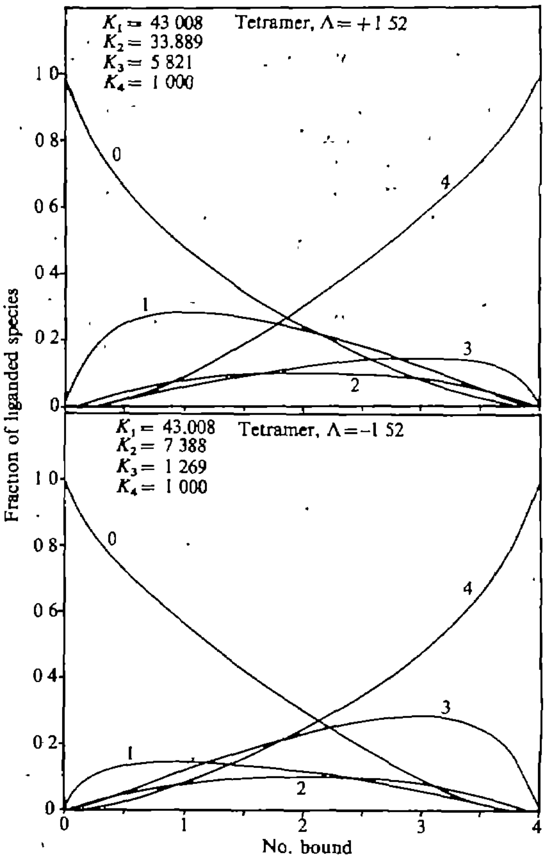

Fig. 2 Skewing in opposite senses of the distribution of ligands for the tetramers of Fig. 1. Numbers on the curves are J values.

and for second order constraints,

$$\Lambda_2 = 2(\bar{\alpha} - \bar{\beta})[f_{\alpha\alpha\beta} - f_\alpha] + [\{\alpha\alpha\} - \{\beta\beta\}](2f_{\alpha\alpha\beta} - 1) \quad (18)$$

and with the substitutions $\delta I = \bar{\alpha} - \bar{\beta}$, $\delta D \doteq \{\alpha\alpha\} - \{\beta\beta\}$ and the f values of equations (16) and (17)

$$\Lambda_1 = \frac{(2\delta I + \delta D)e^{\delta I}(e^{\delta D} - 1) + \delta D(e^{2\delta I + \delta D} - 1)}{(1 + e^{\delta I})(1 + e^{\delta I + \delta D})} \doteq -\Lambda_2 \quad (19)$$

From the last equation,

$$\Lambda_1 = \Lambda_2 = 0 \quad \text{if } \delta D = 0 \quad (20)$$

Therefore, it is a necessary condition for asymmetric ligation, that $\delta D \neq 0$; the 'diagonal' intersubunit couplings $\{\alpha\alpha\}$ and $\{\beta\beta\}$, therefore, must be different. The difference in affinity of the α and β subunits cannot by itself give rise to asymmetry of ligation. If $\delta I = 0$

$$\Lambda_1 = -\Lambda_2 = \delta D \frac{(e^{\delta D} - 1)}{(e^{\delta D} + 1)} \quad (21)$$

and in this case Λ_1 is always positive and Λ_2 always negative. Figure 4 shows plots of Λ_1 and Λ_2 against δD for fixed values of δI. If δI and δD have the same sign Λ_1 is always positive, Λ_2 always negative. Λ_1 can be negative and Λ_2 can be positive if δI and δD have opposite signs and additionally $|\delta D| < |2\delta I|$, the inversion of sign taking place at the point $\delta D + 2\delta I = 0$. A difference in the intrinsic chemical affinity of the subunits can therefore combine with the difference in the value of the diagonal constraints to modulate the asymmetry and even change its sign. From the plots in Fig. 4, as well as from intuitive considerations, one expects that for constraints of the order of 1.5, that is when ligation at the two sides of the boundary decreases the constraints by equal amounts, all asymmetry should disappear, and this can be easily proved by calculating the value of Λ for this case using the method used to derive the values of Λ_1 and Λ_2.

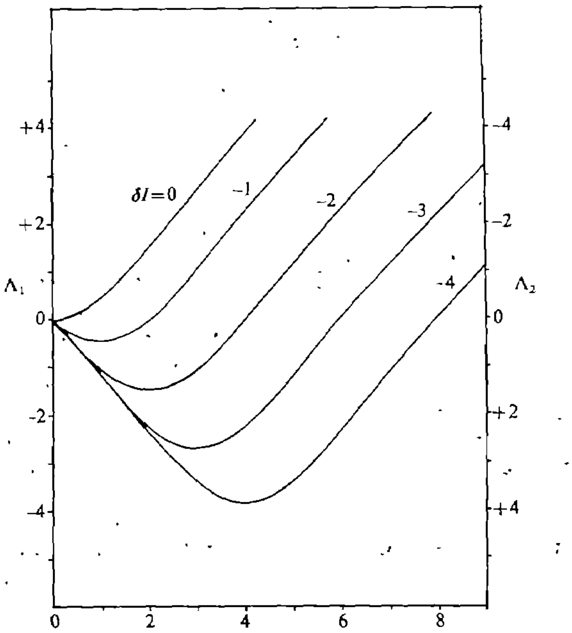

Fig. 4 Plots giving the asymmetry Λ as a function of δD for various values of δI (equation (19)). Λ_1, scale for first order constraints; Λ_2, scale for second order constraints.

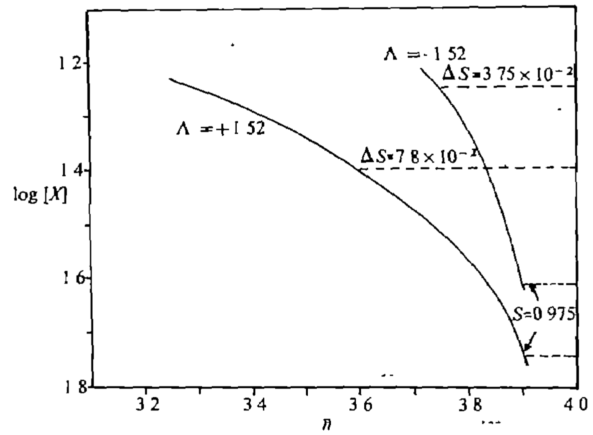

Fig. 3 Differences in the last portion ($\bar{n} > 3.2$) of the Bjerrum plots of the tetramers of Fig. 1, showing the difference in change in saturation (ΔS) for the same increment in free-ligand concentration ($\Delta \log X$).

Application to haemoglobin

The dissociation constants for the four steps of the reaction with oxygen have been determined by Tyuma and coworkers[8,9] for stripped haemoglobin and for the complexes of haemoglobin with diphosphoglycerate (DPG), adenosine triphosphate (ATP) and inositolhexaphosphate (IHP). Knowles and Gibson[10] determined them in the course of a detailed study of the equilibrium of oxygen with haemoglobin in 0.1 M phosphate solutions. All these studies, summarized in Table 1, indicate a high degree of positive asymmetry for the solutions in phosphate or phosphoric esters and a still positive, though clearly a much smaller one for stripped haemoglobin. The increase in positive asymmetry on addition of DPG is no small effect that requires the exact determination of the dissociation constants for its verification. It is evident in the Bjerrum plots of Fig. 3 of the early paper of Benesch *et al.*[11] and in the Hill plots of Fig. 3 of the paper of Tyuma *et al.*[9]. A glance at Fig. 4 indicates that an asymmetry of +3.5 to +4.5 requires a very large difference in the affinities of the α and β chains if the binding constraints are of second order, but a small or null value of δI if the constraints are of the first order. The difference in affinity of the chains is smaller than 1 RT according to Brunori *et al.*[12], and on these grounds it seems safe to conclude that the binding constraints operative in haemoglobin are first order constraints. In calculating the binding constraints from the dissociation constants we found it expedient to set $\delta I = 0$, because the small difference in oxygen affinity of the isolated chains would result in only a minor increase—smaller than 20%—in the value of δD required to satisfy a given asymmetry. Because of the negligible $\alpha\alpha$ boundaries we additionally set $\{\alpha\alpha\} = 0$ or $|\delta D| = \{\beta\beta\}$. With these assumptions the value of the $\{\beta\beta\}$ constraint may be calculated from the asymmetry by means of equation (21) or estimated with acceptable accuracy from the plots of Fig. 4. The total value of the constraints equals the difference between the free energy of binding of the four ligands in the actual system and the free energy of binding to four unconstrained sites:

$$G_4 - G_0 - 4 \ln K_4 = \ln (K_1 K_3 K_2 / K_4^3) = 2S + \{\beta\beta\} \quad (22)$$

The separate symmetric constraints can be determined by fitting the values of G_0 to G_4 to satisfy the experimental quantities. From independent, mainly structural, evidence[13] we assigned the larger of the two values to the $\alpha_1\beta_1$ boundaries (Table 1). While we dare not make an estimate of the uncertainty of the derived constraints, there is no reason to doubt the validity of the general trends. The $\{\beta\beta\}$ constraint increases systematically in the order: stripped Hb < ATP-Hb < PO_4-Hb < DPG-Hb < IHP-Hb, in agreement with observations that indicate the cross-linking of the isolated β chains by IHP[14]. The symmetric constraints are affected in opposite directions by the increase in the $\beta\beta$ constraint. The value of $\alpha_1\beta_1$ increases, but that of $\alpha_1\beta_1$ decreases to the point that in the IHP complexes this boundary is stabilized, not weakened by ligation. Evidently we are far from the simplicity of two-state models. The recent analysis of mutants of haemoglobin has led Pettigrew *et al.*[15] to conclude, with good evidence, that the $\alpha_1\beta_1$ boundary provides the main constraint to binding and is responsible for the greater part of the cooperativity. This conclusion is supported by the data of

Table 1, which, however, indicates that other constraints, particularly the asymmetric one must play a part. An estimation of the binding asymmetry of mutant haemoglobins seems highly desirable.

I shall end by pointing out those aspects of the function of haemoglobin that can only be adequately explained by reference to asymmetric ligand binding. One is the exclusive correlation of the asymmetry with the diagonal constraint, while the greater part of the affinity and cooperative behavior are determined by the symmetric constraints. This separation of functions in different regions of the molecule entails their independence in physiological control and genetic selection. An α_4 tetramer could not be expected to exhibit differences in the diagonal constraints so that the structure of haemoglobin as an $\alpha_2\beta_2$ tetramer appears now justified on functional grounds. To our knowledge no satisfactory reason for this structure has been previously offered.

In haemoglobin the asymmetry increases in the order: stripped Hb < ATP-Hb < PO_4-Hb < DPG-Hb < IHP-Hb, indicating by itself the importance of this property in the molecular adaptation to lower oxygen pressures. The role of the effector in these cases has been seen as one of reducing the affinity for oxygen but some place must be given to the increase in positive asymmetry as this results in a larger unloading of oxygen for the same decrease in partial oxygen pressure. In the absence of differences in the intrinsic ligand affinities of the α and β chains preferential binding to either chain can only take place if there is appreciable asymmetric binding. At the values of Λ of stripped haemoglobin there can only be a slight precedence of α over β in the order of binding, but this precedence will rapidly increase with the $\beta\beta$ cross-linking by the phosphoric effectors. At the asymmetry reached with IHP almost complete precedence of α over β may be expected so that the oxygen transport by haemoglobin will result, to a large extent from reversible binding to the β subunit. The work of Chien Ho and his collaborators[16,17] employing high resolution NMR substantiates this expectation. Considerations of binding asymmetry make clear why haemoglobin is not a suitable model for the regulatory properties of enzymes: haemoglobin works as an easily saturable carrier shuttling between 2/3 and full saturation while enzymes, working more often than not at substrate concentrations that result in modest degrees of ligation, would be expected to benefit from negative binding asymmetry. This could also be at the basis of the often reported partial, or half-of-sites, reactivity[18]. The importance of negative binding asymmetry in these and other cases must await a detailed analysis to be carried out with this precise question in mind.

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Activation of a cellular oncogene by DNA rearrangement: possible involvement of an IS-like element

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The cellular oncogene c-mos *is rearranged in a mouse myeloma and the tumour mRNA contains transcripts hybridizing with a* v-mos *probe. The rearranged gene* (rc-mos) *was cloned in λ phage and shown to transform mouse fibroblasts in transfection assays.* rc-mos *differs from its progenitor,* c-mos, *only at the 5′ end of the gene, where* c-mos *sequences have been substituted by a novel cellular DNA fragment. This fragment contains a 159-base pair (bp) insertion sequence (IS)-like element localized immediately 5′ to the junction with* c-mos. *This is the first demonstration in a non-virally-induced tumour of activation of a cellular oncogene by a mechanism possibly involving DNA transposition.*

THE nature of the genetic events associated with the malignant transformation of normal cells in natural or induced conditions is little understood. Much of our knowledge of these events has come from studies of the rapidly transforming and slowly transforming retroviruses. It is now well established that the first group of viruses acquired their oncogenic properties by incorporating within their genomes one of a group of cellular *onc* (c-*onc*) genes[1,2] which are limited in number[3], evolutionarily conserved[4,5] and probably have an essential physiological role in the normal cell and organism. Once incorporated into the viral genome, the *onc* gene (now termed v-*onc*) is controlled by viral signals and expressed in abnormally elevated levels within the infected cell. It is also possible that the viral *onc* gene product differs in subtle but critical ways from its progenitor, the cellular gene. The slowly transforming retroviruses, which do not transduce cellular genes, were shown in some cases[6,7] to activate transcription of a particular cellular *onc* gene by integration in an adjacent position on the cellular DNA.

The situation is more obscure for the non-virally-induced tumours. By using the DNA-mediated gene transfer approach, it was possible to assay tumour DNAs for their ability to transform mouse fibroblasts in culture. This approach led to the identification of transforming genes in chemically- and radiation-induced tumours[8–12] and in spontaneous human and mouse tumours[9,10,13–16]. Recently, three transforming genes from human bladder carcinomas[17–19] and one human lung carcinoma[17] were identified as the activated forms of the normal human homologue of the viral *onc* genes Ha-*ras* and Ki-*ras*, respectively. Preliminary attempts using restriction enzyme mapping were unable to detect structural differences between the human transforming genes and their normal counterparts[18,19]. On the other hand, the association of chromosomal aberrations with many murine and human tumours suggested that DNA rearrangements might constitute a general mechanism for tumour induction[20], possibly by their effect on cellular oncogenes[21].

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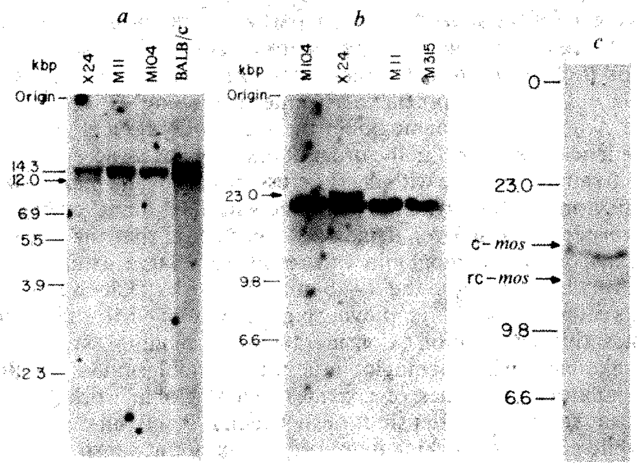


Fig. 1 Analysis of the c-*mos* gene in mouse myeloma DNAs. High molecular weight DNA from mouse myelomas MOPC104E, XRPC24, MPC11 and MOPC315 (referred to in the figure as M104, X24, M11 and M315, respectively) was digested with *Eco*RI (*a*) or *Bam*HI (*b*). Aliquots (20 µg) were electrophoresed on 0.7% agarose gels, blotted onto nitrocellulose filters and hybridized to the *Hin*dIII–*Bgl*I v-*mos*-specific fragment[23] radiolabelled by nick-translation. After hybridization, the blots were subjected to autoradiography. For molecular cloning, the c-*mos* and rc-*mos* *Eco*RI DNA fragments were enriched from XRPC24 DNA by electrophoresing 500 µg of digested DNA on 0.5% agarose gel, identifying the location of the two fragments on the gel by hybridization (*c*) and elution of the DNA by adsorption to glass beads[50]. Arrows indicate the rearranged band.

We undertook the present studies to search in non-virally-induced murine myelomas for the possible activation of another cellular oncogene, c-*mos*. This gene, which is the progenitor of the transforming information in Moloney murine sarcoma virus (M-MuSV)[22,23], was characterized extensively by transfection studies[23-25] and nucleotide sequencing analysis[26,27]. These studies established that the cellular information contained within the genome of M-MuSV (denoted v-*mos*), and presumably transduced out of the mouse genome during generation of this virus, consists of a long open reading frame of 369 codons. This sequence is joined in phase to a segment of five codons of viral helper sequence immediately 5′ to the v-*mos* region[26,27]. A 37,000-molecular weight (MW) protein identified within virus-infected cells[28] is presumably encoded by the 374-codons sequence and directly triggers cellular malignant transformation. The cellular homologue of v-*mos* (c-*mos*) contains 21 more codons joined in phase 5′ to the 369-codon open reading frame described above[27]. So far, no proteins encoded by this gene have been found in normal cells. Here we show that the c-*mos* gene is biologically activated in a particular mouse myeloma. The activation is due to DNA rearrangement at the 5′ end of the cellular gene, involving deletion of c-*mos* information and insertion of a small DNA fragment having structural properties characteristic of an IS element.

c-*mos* gene is rearranged and transcribed in a mouse myeloma

We screened the DNA of several BALB/c mouse myelomas by Southern blot analysis using the *Hin*dIII–*Bgl*I *src*-specific fragment of v-*mos*[23] as a probe; one of the tumours—XRPC24—showed an additional hybridizing band detected by this probe (Fig. 1). In the *Eco*RI digest, this additional band was smaller (12.5 kilobase pairs, kbp) than the 14-kbp c-*mos* band, whereas in the *Bam*HI digest the additional band was larger (23 kbp) than the 21-kbp c-*mos* band. These results indicated that the endogenous c-*mos* gene had undergone some rearrangement in the pristine-induced XRPC24 tumour, therefore we will refer to it as rc-*mos*. We attempted to determine whether this DNA rearrangement is associated with enhanced transcription of the gene. The poly(A)-containing RNA fraction was extracted from the same plasmacytomas and analysed by the Northern technique for *mos*-specific transcripts. Figure 2 shows that the probe detected a transcript of 1.2 kilobase (kb) in the RNA of myeloma XRPC24. No hybridization was observed with RNAs from the other tumours tested. Thus, the appearance of rc-*mos* DNA is correlated with increased transcription of the gene.

To compare c-*mos* and rc-*mos*, we cloned both genes from myeloma XRPC24 DNA digested with *Eco*RI. The 14-kbp and 12.5-kbp *mos* fragments were enriched by preparative agarose gel electrophoresis (see Fig. 1*c*) and cloned into Charon 4A λ phage vector[29]. Several clones containing one of the two inserts were purified and analysed in detail.

c-*mos* and rc-*mos* differ at the 5′ region

The two cloned genes were analysed by restriction enzymes; the partial physical maps are shown in Fig. 3. The restriction map of c-*mos* is identical to that published by Vande Woude and co-workers[25]. rc-*mos* shares with c-*mos* an identical 12-kbp fragment between the *Bgl*I site at 3.1 kbp on the map and the 3′ end of the insert. The two clones differ at the region 5′ to the *Bgl*I site up to the *Eco*RI site. The sequence of this region in the rc-*mos* clone, determined according to the strategy shown

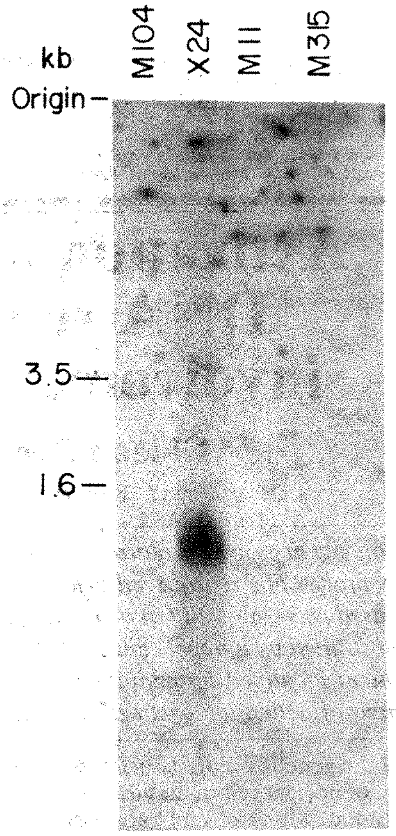


Fig. 2 Analysis of poly(A)$^+$ RNA from mouse myeloma for the presence of c-*mos* transcripts. Aliquots (50 µg) of poly(A)-selected RNA from myelomas MOPC315, MOPC104E, XRPC24 and MPC11 (M315, M104, X24, M11 in the figure) were heated at 60 °C for 10 min in a solution containing 50% formamide, 6% formaldehyde and running buffer (20 mM MOPS *p*H 7.0, 5 mM NaAc, 1 mM EDTA). The samples were electrophoresed at 100 V for 5 h in 1% agarose gel containing 6% formaldehyde and 1× running buffer. The RNA was transferred with 10×SSC to nitrocellulose filters, fixed by heating at 80 °C for 2 h and hybridized at 43 °C for 2 days to 2×10^6 c.p.m. ml^{-1} of v-*mos* probe in a solution containing 50% formamide, 5×SSC, 1×Denhardt's mixture, 20 mM sodium phosphate *p*H 7.0, and 100 $\mu\mathrm{g\,ml}^{-1}$ salmon sperm DNA. After washing at 50 °C with a solution of 0.1×SSC, 0.1% SDS, the filters were autoradiographed. Kb, kilobase.

in Fig. 3, is shown in Fig. 4 together with the corresponding published sequence of c-*mos*[27].

Clearly, rc-*mos* and c-*mos* are identical 3′ to nucleotide 513 and completely divergent 5′ of this point. The c-*mos* sequence deleted in the rearranged gene includes about one-quarter of the presumed c-*mos* coding sequence[27]. The deleted sequence also includes the first 41 codons in the portion of the gene shown to be conserved in both man and mouse[30]. The inserted novel DNA fragment contains an open reading frame of 28 codons which joins in phase the rest of the c-*mos* open reading frame at nucleotide 513 in the sequence shown in Fig. 4.

Unique structural features of the novel DNA sequences identified in rc-*mos*

Analysis of the rc-*mos* sequence immediately 5′ to the junction point (Fig. 4) showed the hexanucleotide CCAACA at positions 507–512. An almost perfect inverted repeat of this sequence—TGATTGG—was found 153 nucleotides upstream at positions 354–360. Further inspection of the sequence indicated that the 159-bp fragment terminated with the inverted repeats, is bound on both sides at positions 347–352 and 513–519 by the hexanucleotide, CACTCC, in a configuration of a direct repeat. As this hexanucleotide is part of the c-*mos* sequence, it must have duplicated during the insertion of the novel 159-bp fragment. Presumably, concomitant or subsequent to this insertion, c-*mos* sequences 5′ to the insertion point were deleted. The fragment encompassing the inverted and direct repeats can be drawn in a typical stem-and-loop configuration (see Fig. 5). It is remarkable that the 159-bp fragment ends with the dinucleotide TG…CA as do all eukaryotic movable elements[31–33], Mu phage[34,35] and retroviruses[36–38]. Finally, the 3′-terminal pentanucleotide of the fragment, CAACA, is identical to the 3′ terminus of the *Drosophila* transposable element *copia*[33], yeast Ty*1* mobile element[31,32], and spleen necrosis virus DNA[36].

Biological activity of the rearranged c-*mos* gene

Previously, it was shown that the molecularly cloned, 14-kbp c-*mos* gene was inactive when assayed for its ability to transform NIH 3T3 cells in transfection experiments[25]. To demonstrate biological activity of the gene, it was necessary to ligate a viral long terminal repeat (LTR) upstream from the 5′ end of the gene[25]. We asked whether the rearranged c-*mos* gene cloned from myeloma XRPC24 is active by itself when assayed by transfection; a typical experiment is shown in Table 1.

The two clones of the rc-*mos* gene that were tested induced a large number of foci having specific infectivities of 3,100 and 4,900 focus-forming units (FFU) per μg DNA, respectively (Table 1). In contrast, clones of the 14-kbp DNA fragment containing the normal c-*mos* gene showed negligible or no activity. As a positive control we used two recombinant phages containing integrated Moloney sarcoma viral DNA (E.C., unpublished) which produced 10,200 and 13,200 FFU per μg DNA (Table 1).

The cells transformed by rc-*mos* DNA were indistinguishable morphologically from those transformed with the viral genome. The transformants consisted of round and spindle-shaped highly refractile cells which grew in multilayers. The foci induced by rc-*mos* DNA could be detected 12 days after transfection in contrast to the 10 days required to detect the viral genome-induced transformants.

Conclusions

Here we have described a genetic event which led to the activation of a cellular *onc* gene in a non-virally-induced tumour. The normal c-*mos* gene has undergone DNA rearrangements in the murine myeloma XRPC24 and has become highly potent in transforming mouse fibroblast monolayers. The biological activation was associated with the appearance of c-*mos* gene transcripts in the tumour tissue. This is all the more notable as, unlike many cellular *onc* genes which are expressed in various normal and malignant cells[39–42], c-*mos* gene transcripts have not been detected previously in all cells examined[43]. The rearrangement of the gene was twofold: first, all sequences 5′ to position 513 (Fig. 4), including 263 nucleotides of the presumed coding sequence, were deleted; second, this information was substituted by a novel cellular DNA fragment. Within this fragment, the 3′-terminal 159-nucleotide sequence demonstrated several features (see Fig. 5) in common with bacterial IS elements[44,45], Mu phage[34,35], eukaryotic transposable elements[31–33] and retroviruses[36–38]: (1) Like all these elements, the sequence ends in a terminal inverted repeat. (2) The 3′-terminal two nucleotides, CA, and the 5′-terminal TG are common to all eukaryotic cellular movable elements, Mu phage and retroviruses. (3) The 3′-terminal five nucleotides, CAACA, are shared with the terminal sequence of yeast Ty*1* and *Drosophila copia* elements as well as with spleen necrosis virus DNA. (4) The sequence is bound on both sides by a direct repeat derived from c-*mos* DNA at the junction site. Similar short sequence duplications of the integration site are characteristic of all elements described above. Because of the small size of the sequence found here—159 bp—and the fact that it does not consist of two long terminal direct repeats, we consider the element to resemble most strongly bacterial IS elements or the Ty*1*-related solo δ units found in yeast[46]. Inspection of the sequence reveals neither transcription initiation and termination signals characteristic of retroviral long terminal repeats, nor any other homology to these viral sequences. By using hybridization methods, we detected no sequence homology between the DNA segment inserted 5′ to c-*mos* and Moloney murine leukaemia viral DNA. Moreover, our preliminary results indicate that although sequences within this DNA segment are present in normal mouse cell DNA in multiple copies, unlike endogenous murine retroviral sequences[47], they distribute in a highly conserved pattern in different mouse strains.

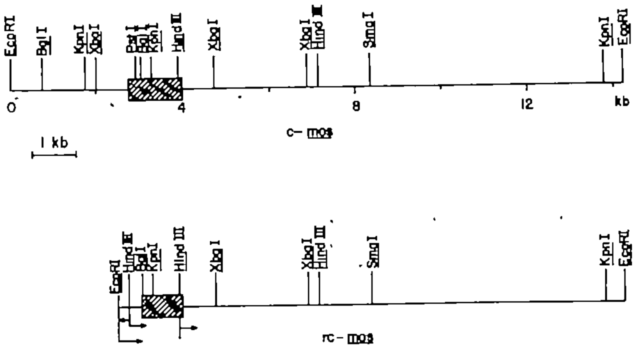
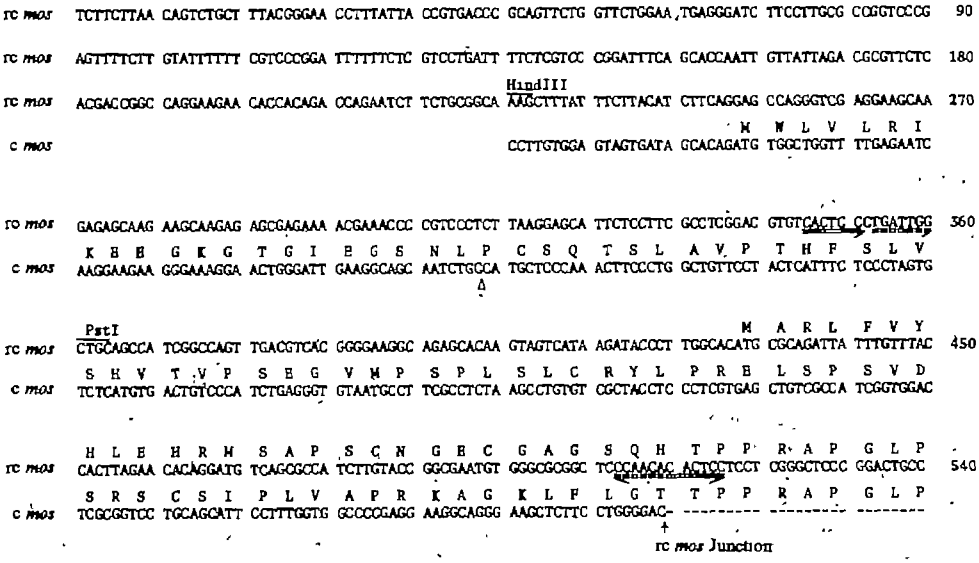


Fig. 3 Physical map of rc-*mos* and c-*mos*. The arrows indicate the strategy for DNA sequencing of rc-*mos*.

Table 1 Biological activity of molecularly cloned c-*mos* and rc-*mos* DNAs

Clone	Insert DNA (ng)	No. of foci	Specific infectivity (FFU per pmol DNA)
λ-MSV 26	50	150	10,200
λ-MSV 3-3	100	220	13,200
λ-c-*mos* IA	125	0	67
λ-c-*mos* IB	125	1	<67
λ-rc-*mos* 2B	100	43	3,100
λ-rc-*mos* 2F	100	68	4,900

Intact recombinant phage DNA was transfected into NIH 3T3 fibroblasts by the calcium phosphate method[49]. The cultures were kept in medium containing 5% fetal calf serum and were scored for focus formation 2 weeks after transfection. λ-MSV 26 and λ-MSV 3-3 represent M-MuSV molecules integrated within normal rat kidney cell DNA and molecularly cloned in λ Wes B vector. λ-c-*mos* and λ-rc-*mos* are molecular clones of the c-*mos* and rc-*mos* genes, respectively, obtained from XRPC24 myeloma DNA.



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                                                                        HindIII
rc mos  TCTTCTTAA CAGTCTGCT TTACGGGAA CCTTTATTA CCGTGACCC GCAGTTCTG GTTCTGGAA TGAGGGATC TTCCTTGCG CCGGTCCCG   90

rc mos  AGTTTTCTT GTATTTTTT CGTCCCGGA TTTTTTCTC GTCCTGATT TTCTCGTCC CGGATTTCA GCACCAATT GTTATTAGA CGCGTTCTC  180
                                                     HindIII
rc mos  ACGACCGGC CAGGAAGAA CACCACAGA CCAGAATCT TCTGCGGCA AAGCTTTAT TTCTTACAT CTTCAGGAG CCAGGGTCG AGGAAGCAA  270
                                                                                  M  W  L  V  L  R  I
c mos                                                CCTTGTGGA GTAGTGATA GCACAGATG TGGCTGGTT TTGAGAATC


rc mos  GAGAGCAAG AAGCAAGAG AGCGAGAAA ACGAAACCC CGTCCCTCT TAAGGAGCA TTCTCCTTC GCCTCGGAC GTGTCACTC CCTGATTGG  360
         K  E  E   G  K  G   T  G  I   E  G  S   N  L  P   C  S  Q   T  S  L   A  V  P   T  H  F   S  L  V
c mos   AAGGAAGAA GGGAAAGGA ACTGGGATT GAAGGCAGC AATCTGGCA TGCTCCCAA ACTTCCCTG GCTGTTCCT ACTCATTTC TCCCTAGTG
                                                 Δ

         PstI                                                                M  A  R   L  F  V  Y
rc mos  CTGCAGCCA TCGGCCAGT TGACGTCAC GGGGAAGGC AGAGCACAA GTAGTCATA AGATACCCT TGGCACATG CGCAGATTA TTTGTTTAC  450
         S  H  V   T  V  P   S  E  G   V  M  P   S  P  L   S  L  C   R  Y  L   P  R  E   L  S  P   S  V  D
c mos   TCTCATGTG ACTGTCCCA TCTGAGGGT GTAATGCCT TCGCCTCTA AGCCTGTGT CGCTACCTC CCTCGTGAG CTGTCGCCA TCGGTGGAC

         H  L  E   H  R  M   S  A  P   S  C  N   G  E  C   G  A  G   S  Q  H   T  P  P   R  A  P   G  L  P
rc mos  CACTTAGAA CACAGGATG TCAGCGCCA TCTTGTACC GGCGAATGT GGGCGCGGC TCCCAACAC ACTCCTCCT CGGGCTCCC GGACTGCC   540
         S  R  S   C  S  I   P  L  V   A  P  R   K  A  G   K  L  F   L  G  T   T  P  P   R  A  P   G  L  P
c mos   TCGCGGTCC TGCAGCATT CCTTTGGTG GCCCCGAGG AAGGCAGGG AAGCTCTTC CTGGGGAC- --------- --------- --------
                                                                   ↑
                                                            rc mos Junction
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Fig. 4 Sequence of the novel DNA segment inserted into c-*mos*. The fragment encompassing the *Eco*RI–*Bgl*I region at the 5′ end of the rc-*mos* gene (Fig. 3) was sequenced according to Maxam and Gilbert[51]. Arrowed lines indicate the position of the direct (solid line) and inverted (broken line) repeats at the borders of the IS-like element. The c-*mos* sequence is shown for comparison, beginning with the first ATG of the open reading frame[27]. Amino acids translated from the DNA sequence are given in the single letter code. The junction between the novel DNA fragment and the c-*mos* sequence is indicated by a small arrow. The junction[26] between the viral helper DNA and the *mos* information within the M-MuSV genome (v-*mos*) is indicated by an open triangle. The sequence numbers are from the *Eco*RI site shown in Fig. 3.

Finally, note that the mechanism by which the DNA rearrangement took place is probably very complex. The removal of c-*mos* sequences 5′ to the insertion point suggests a process of insertion followed by deletion.

The mechanism by which the substitution of c-*mos* sequences with the new DNA fragment activated the gene is unclear. It is probable that the increased transcription of the gene is directly related to the change in its biological properties. It is also possible that the alterations in the structure of the putative protein encoded by the c-*mos* gene, had a rôle in the activation. It is tempting to speculate that the IS-like element inserted in the gene directly triggers the activation. The element could initiate transcription of the c-*mos* gene by introducing a strong promoter[48]. Alternatively, the presence of the element may lead to modifications of the chromatin structure at this site, which could create a putative promoter within the adjacent c-*mos* gene available for transcription. Analysis of the RNA transcripts, together with transfection studies using different DNA fragments, should elucidate the mechanism of activation.

The finding of a cell line synthesizing mRNA (and probably a transforming protein) encoded by the c-*mos* gene, albeit modified, provides the first opportunity of studying *in vivo* transcription of the cellular gene. The ability of the modified gene to transform suggests that the 263 nucleotides at the 5′ end of the c-*mos* gene as well as the 5′ 67 codons of v-*mos*, are dispensable for the transforming activity.

c-*mos* represents the third c-*onc* gene after Ha-*ras* and Ki-*ras*[17–19], shown to be activated in a non-virally-induced tumour. The modification here involved rearrangement at the 5′ end of the gene, including insertion of a 159-bp DNA fragment having the structure of an IS element. This is the first example of oncogene activation in a non-virally-induced tumour by a process of DNA transposition. Whether this is unique, or represents a general method of malignant conversion of potentially transforming cellular genes remains to be shown.

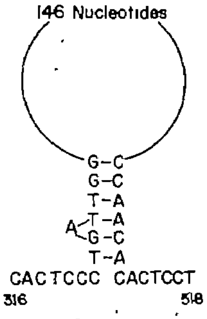

Fig. 5 Schematic representation of the IS-like element present at the rc-*mos* junction. Numbers are as in the nucleotide sequence given in Fig. 4.

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Note added in proof: Rearrangement of cellular oncogenes might not be a very rare event, since we have now found that the c-*myc* gene has undergone rearrangements in several mouse plasmacytomas. In addition, we have recently obtained evidence for the rearrangement of the human c-*mos* gene in a particular Burkitt lymphoma line. In this case the rearranged c-*mos* gene was inserted near an immunoglobulin V_H gene. This rearrangement has probably resulted from the translocation of the end of chromosome 8, where human c-*mos* is located, to chromosome 14, where the human immunoglobulin heavy chain genes reside.

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Dramatic growth of mice that develop from eggs microinjected with metallothionein–growth hormone fusion genes

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A DNA fragment containing the promoter of the mouse metallothionein-I gene fused to the structural gene of rat growth hormone was microinjected into the pronuclei of fertilized mouse eggs. Of 21 mice that developed from these eggs, seven carried the fusion gene and six of these grew significantly larger than their littermates. Several of these transgenic mice had extraordinarily high levels of the fusion mRNA in their liver and growth hormone in their serum. This approach has implications for studying the biological effects of growth hormone, as a way to accelerate animal growth, as a model for gigantism, as a means of correcting genetic disease, and as a method of farming valuable gene products.

THE introduction of foreign genes into mammalian embryos could form the basis of a powerful approach for studying gene regulation and the genetic basis of development. Recent studies have clearly established the feasibility of introducing foreign DNA into the mammalian genome by microinjection of DNA molecules of interest into pronuclei of fertilized eggs, followed by insertion of the eggs into the reproductive tracts of foster mothers[1–7]. The data suggest that the so-called transgenic animals that develop from this procedure integrate the foreign DNA into one of the host chromosomes at an early stage of embryo development. As a result, the foreign DNA is generally transmitted through the germ line[3,8,9]. In some cases, expression of these foreign genes has also been detected; low levels of rabbit β-globin and viral thymidine kinase (TK) activity were measured in transgenic mice after introducing the respective genes[6,7]. It is possible to obtain regulated expression of viral TK by fusing the structural gene to the mouse metallothionein-I (MT-I) gene promoter and inducing expression with heavy metals[2,9].

The possibility of introducing genes encoding secreted regulatory peptides into animals via this technology has several potential applications. The growth hormone (GH) gene whose protein product has profound developmental effects is particularly convenient for testing this possibility. Based on our success in achieving regulatable expression of herpes TK using the MT-I promoter[9], we fused this promoter to the rat GH structural gene which has been shown to direct GH synthesis in mouse cells[10]. We show here that when this gene construct (MGH) is introduced into eggs it is expressed efficiently, giving rise to greater than normal levels of growth hormone in transgenic mice. The most obvious consequence of this high level of growth hormone expression is gigantism. This success raises several issues of genetic and practical importance.

Generation of animals carrying MGH fusion genes

A fragment of the cloned rat GH gene, from which the 5′ regulatory region had been deleted, was fused to the MT-I promoter region, generating the plasmid pMGH (see Fig. 1*a*). In this construction, the fusion occurs in exon 1, retaining the initiation codon of the GH gene and the 5′ regulatory region of the MT-I gene. Thus, the fusion gene is predicted to direct transcription of a mRNA containing 68 bases contributed by MT-I, followed by 1 base contributed by an *Xho*I linker, then the rat GH mRNA sequence starting at nucleotide 7. This construction preserves the AUG initiation codon for GH synthesis located at position 124–126 (Fig. 1*c*), the four intervening sequences and the poly(A) site of the rat GH gene (Fig. 1*b*), and 3 kilobases (kb) of downstream chromosomal sequences.

A 5.0-kb fragment extending from the *Bgl*I site of MT-I (−185) to *Bam*HI (see Fig. 1*b*) was restricted from pMGH, separated from the other fragments on an agarose gel and used for injection into eggs. This linear fragment with heterologous ends was chosen because our recent experience suggests that such fragments integrate into host DNA more efficiently than supercoiled plsmids. Although this fragment contains only 253 base pairs (bp) of MT-I sequence, we have shown previously that this includes sequences essential for heavy metal regulation of the MT-I promoter[11,12]. The male pronuclei of fertilized eggs were microinjected with 2 pl containing ~600 copies of this fragment and 170 eggs were inserted into the reproductive tracts of foster mothers; 21 animals developed from these eggs[2].

When they were weaned, total nucleic acids were extracted from a piece of tail and used for DNA dot hybridization to

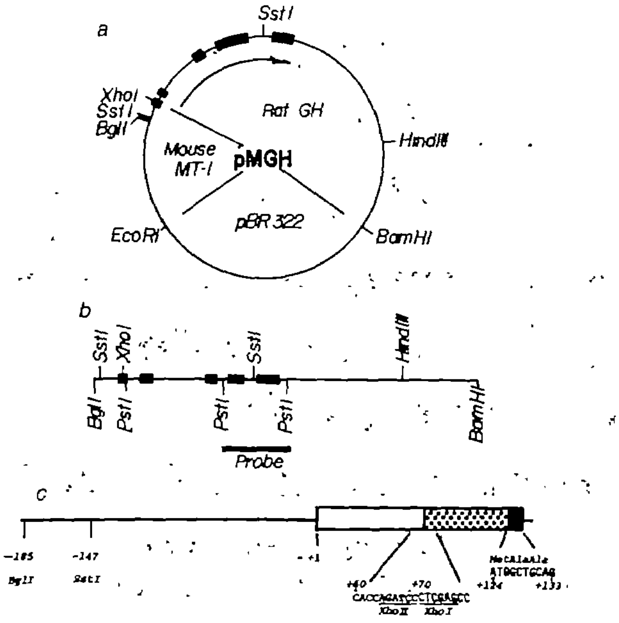

Fig. 1 Construction of the fusion plasmid pMGH. *a*, The unique *Bgl*II site of the MT-I genomic clone[28] was converted to a *Xho*I site by digesting with *Bgl*II, followed by filling in the sticky ends with Klenow fragment of DNA polymerase in the presence of all four dNTPs; then *Xho*I linkers (CCTCGAGG) were ligated to the blunt ends and bacterial strain RR1 was transformed with this DNA. The *Pvu*II site of pBR322 was converted to a *Bam*HI site by a similar procedure, then the 4.1-kb *Xho*I–*Bam*HI fragment containing the MT-I promoter and pBR was ligated to a 4.8-kb *Xho*I–*Bam*HI fragment containing the rat GH structural gene (see refs 10, 30) to give pMGH (8.9 kb). *b*, The fragment used for injection was a 5.0-kb *Bgl*I–*Bam*HI fragment which was isolated from an agarose gel by the NaClO$_4$ method[29]. For genomic Southern blots, a 1.0-kb *Pst*I fragment spanning exons 4 and 5 was isolated and nick-translated[9] and used as a hybridization probe. *c*, The predicted structure of exon 1 of the fusion gene. The line and open box represent MT-1 untranslated sequences, the stippled box represents GH untranslated sequences and the solid box represents the beginning of the GH coding region (see refs 28 and 30 for complete sequence information).

determine which animals carried MGH sequences. Seven of the animals gave hybridization signals above background (Fig. 2*a*) and their DNA was analysed further by restriction with *Sst*I and Southern blotting. This analysis showed that all seven had an intact 1.7-kb *Sst*I fragment predicted from the restriction map shown in Fig. 1. All the animals having a prominent 1.7-kb hybridizing band also revealed a 3.3-kb band. This band is predicted from a circularized version of the 5.0-kb *Bgl*I–*Bam*HI fragment. Two of the mice having multiple copies (MGH-10 and -19) gave a 5.0-kb hybridizing fragment when digested with *Hin*dIII, an enzyme that cuts only once within the injected fragment. Surprisingly, *Bam*HI also generates a 5.0-kb hybridizing fragment in all the DNAs analysed (MGH-10, -14, -16 and -19), suggesting that this restriction site was restored during circularization, whereas *Bgl*I and *Eco*RI (which does not cut within the fragment) gave larger fragments (data not shown). We conclude that all the MGH-positive animals have at least one intact *Bgl*I–*Bam*HI insert and four of the mice have a tandem head-to-tail duplication of this 5.0-kb fragment; however, the details of how and when this fragment circularized and integrated are not discernible. One of the mice, MGH-10, transmitted the MGH genes to half (10 of 19) of its offspring, suggesting that these genes are stably integrated into one of its chromosomes.

Rapid growth of mice carrying MGH genes

At 33 days post-parturition, all of the mice were weaned and maintained on a solid diet supplemented with water containing 5,000 p.p.m. ZnSO$_4$ (76 mM); their growth rate was recorded periodically. The dose of Zn was chosen on the basis of experiments which indicated that 5,000 p.p.m. induced almost maximal levels of MT-I mRNA in the liver without imparing the breeding potential of mice maintained on this diet for several months. Figure 3 shows the growth of the seven mice carrying MGH sequences. The littermates without MGH sequences served as convenient controls; the weights for each sex were averaged separately. Six of the mice with MGH sequences showed substantially more weight gain (up to 1.8-fold) than the controls. With the exception of MGH-16, there was a correlation between weight gain and MGH gene dosage (Table 1). The animal carrying the most copies of MGH sequences, MGH-21, died after 7 weeks of rapid growth; other mice have, however, grown larger. Most of the animals were already larger than normal before the Zn diet was instituted. Furthermore, one of the animals (MGH-19) was removed from the Zn diet on day 56 and it continued to grow at an accelerated rate. Thus, the effect of Zn on growth rate is uncertain. Perhaps mice carrying several copies of the MGH gene produce excess GH constitutively without a requirement for heavy metal induction. Thus, the question of Zn-dependent growth will be most easily answered by analysis of offspring of mice such as MGH-16 that contain only a few copies of the MGH gene.

Expression of the MGH gene in the liver

Based on previous studies with mice carrying metallothionein–TK fusion genes, maximal expression of MGH was expected in the liver, intestine and kidney. Evaluation of the tissue specificity of MGH gene expression was initiated by analysis of its activity in liver. A partial hepatectomy on day 56 allowed isolation and quantification of RNA for MGH-specific sequences. Figure 4*b* shows the results of RNA slot hybridization. The level of MGH mRNA expression in the liver correlated with the growth of the transgenic mice. The largest animals, MGH-2, -19 and -21, contained large amounts of MGH mRNA in the liver, whereas MGH-10, -14 and -16, which showed slower growth rates, contained ~50-fold less MGH RNA, not visible on the exposure shown in Fig. 4. Analysis of samples exposed to base hydrolysis before spotting the nucleic acids on to nitrocellulose (Fig. 4*a*) or treated with RNases A plus T$_1$ after hybridization (not shown) established that RNA rather than potentially contaminating DNA was hybridizing to the probe. From these data, we estimate that 30 μg of liver RNA from MGH-2, -19 and -21 contains the same amount of GH mRNA as 13, 25 and 50 ng of poly(A)$^+$ rat pituitary RNA,

Table 1 MGH genes, mRNA, GH levels, and growth of transgenic mice

Mouse	Sex	No. of MGH genes per cell	No. of MGH mRNA molecules per cell	Growth hormone (μg ml^{-1})	Growth* (g)	Ratio
MGH-2	♀	20	800	57.0	41.2	1.87
MGH-3	♀	1	<50	0.87	22.5	1.02
MGH-10	♂	8	<50	0.28	34.4	1.32
MGH-14	♂	2	<50	0.31	30.6	1.17
MGH-16	♂	2	<50	17.9	36.4	1.40
MGH-19	♂	10	1,500	32.0	44.0	1.69
MGH-21	♀	35	3,000	112.0	39.3	1.78
Female littermates (*n* = 3)		0	0	0.16 ±0.1	22.0 ±0.8	
Male littermates (*n* = 11)		0	0	0.15 ±0.08	26.0 ±2	

The number of MGH genes per cell was estimated by DNA dot hybridization and scintillation counting (Fig. 2*a*). MGH mRNA molecules per cell were estimated by densitometric scanning of the autoradiogram from RNA slot hybridization (Fig. 4) and assuming 2.5×10^5 mRNA molecules per liver cell. GH was measured by RIA as described elsewhere[10].

* Animal weights when 74 days old. The ratio of body weight compared with littermates of the same sex is indicated (see Fig. 3).

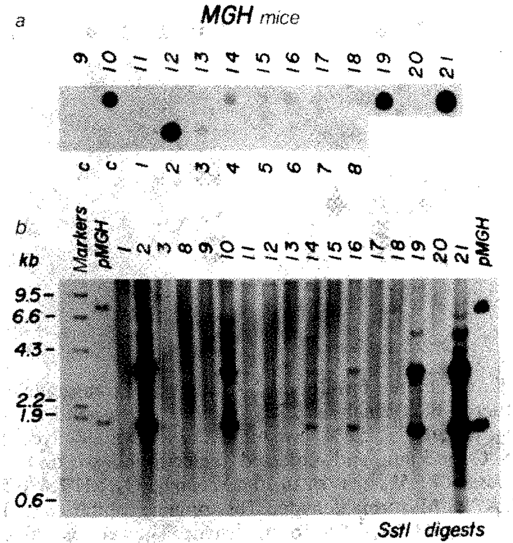

Fig. 2 Analysis of DNA from transgenic mice for MGH sequences. *a*, DNA dot hybridization[9] was used to detect mice with MGH sequences and quantitate their abundance. A nick-translated *Pst*I probe (see Fig. 1*b*) was used (6 h exposure). Numbers correspond to the MGH mice examined; c, controls. *b*, DNA (5 μg) from MGH mice was restricted with *Sst*I electrophoresed on a 1% agarose gel, transferred to nitrocellulose and hybridized with the nick-translated *Pst*I probe shown in Fig. 1*b*. pMGH (13 pg, left and 130 pg, right) was included as a hybridization standard. Markers are *Hin*dIII-cut λ DNA end-labelled with ^{32}P.

respectively. The RNA standard used contains about 10% GH mRNA on the basis of Rot analysis; therefore, we estimate that there are ~800–3,000 MGH mRNA molecules per liver cell in these transgenic mice (Table 1).

If all processing signals in the fusion gene are correctly recognized, a fusion mRNA 63 nucleotides larger than *bona fide* rat GH mRNA (~1 kb) would be generated[10,13]. Denaturing gel electrophoresis and RNA Northern blot analysis of the liver RNA from MGH-21 show that its size is similar, as expected, to that of authentic mouse and rat pituitary GH mRNA (Fig. 5*a*). Liver RNA from a control mouse shows no GH-reactive sequences (Fig. 5*a*, lane 1). Because the GH cDNA probe used for the RNA blot analysis recognizes both rat and mouse GH mRNAs, it is necessary to establish that the hybridizing species in the liver is actually the product of the fusion gene and not mouse GH mRNA due to an unexpected activation of the endogenous mouse GH gene. The use of a *Xho*I linker for the construction of the fusion gene generates a sequence that will be uniquely present in MGH mRNA. Thus, the *Xho*I site of pMGH was labelled using [γ-^{32}P]ATP and polynucleotide kinase, followed by cleavage with *Sst*I (Fig. 1*c*). This 221-nucleotide fragment was gel-purified, denatured, and used as hybridization probe in a single-strand-specific nuclease protection assay. Hybridization to MGH mRNA should generate a 74-base nuclease-resistant fragment while mouse GH mRNA or metallothionein mRNA will be unable to protect the kinased end and should therefore be negative. Figure 5*b*, lane 6 shows that the predicted 74-base fragment is present in liver RNA of mouse MGH-21, but not in normal mouse pituitary RNA (lane 5), control mouse liver RNA (lane 4) or in the liver RNA of MGH-3 (lane 3), an animal showing no accelerated growth (Fig. 3). Thus, it seems that transcription is initiating properly at the MT-I promoter and continuing through the putative termination site of the GH gene, the four GH intervening sequences are being properly spliced and the MGH mRNA is polyadenylated.

Increased growth hormone in sera of MGH mice

The correlation between MGH mRNA levels and growth of the mice sugggests that expression of the MGH gene accounts for the observed biological consequences; this was supported by the three independent RNA analyses above. These results suggest that the circulating levels of GH should be increased. Blood was taken from the transgenic mice and from littermates and assayed for GH by radioimmunoassay (RIA). The GH levels in four of the transgenic mice were 100–800 times greater than levels in control littermates; one mouse had 112 μg ml^{-1} of GH in its serum (Table 1). Two of the transgenic mice showing a slow but significantly elevated growth rate had serum GH levels at the high end of the normal range. The lack of physiological regulation and the ectopic production of GH in these animals probably account for their accelerated growth.

Discussion

These data strongly suggest that the altered phenotype of the mice is a direct result of the integration and expression of the metallothionein–growth hormone fusion gene. The elevated level of GH present in some of these mice corresponds to a high level of MGH mRNA in the liver (up to 3,000 molecules per cell). The accumulation of MGH mRNA in the liver is comparable to the endogenous level of MT-I mRNA[14], but is ~100-fold higher than that obtained from metallothionein–TK fusion genes studied previously[2]. This difference is probably the consequence of the intrinsic stability of the GH mRNA relative to TK mRNA; however, differences in transcription rates or processing efficiency due to variations in fusion gene construction are also possible. The high level of MGH gene expression in transgenic mice will greatly facilitate direct comparison of MGH and MT-I mRNA production in different tissues, thus allowing us to determine whether chromosomal location has an important effect on tissue-specific expression of the MT-I promoter.

Growth hormone levels in some of the transgenic mice were as much as 800-fold higher than in normal mice, resulting in animals nearly twice the weight of their unaffected littermates. This greater than normal accumulation of GH undoubtedly reflects both the lack of normal feedback mechanisms and expression of this gene in many large organs including liver, kidney and intestine. The effect of chronic exposure to high levels of GH is well documented[15], resulting in the clinical condition referred to as gigantism. This condition in humans is

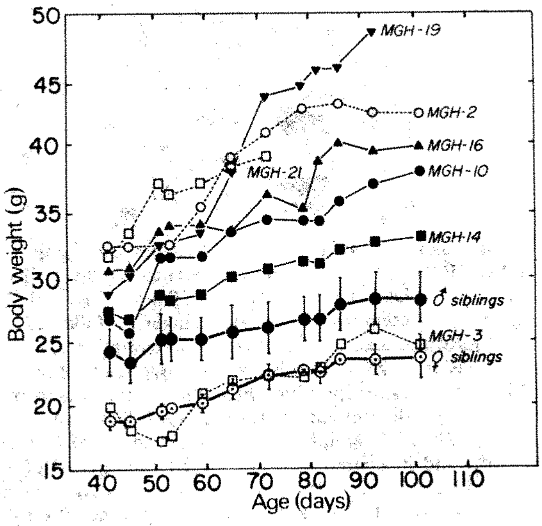

Fig. 3 Growth of MGH mice. Microinjected eggs (SJL×C57) were transferred to oviducts of foster mothers on 14 April; 21 animals were born 3 weeks later. At 33 days old they were weaned and the drinking water was supplemented with 76 mM $ZnSO_4$. The body weights of the males are shown as solid symbols; the mean weight (±s.d.) of 11 siblings not containing MGH sequences is also shown. The female weights are represented by open symbols; means (±s.d.) of three siblings are indicated also. MGH-21 died on day 72. A partial hepatectomy was performed on MGH-19 on day 56 and it was taken off Zn thereafter; it was killed on day 100. All mice were taken off Zn after day 83.

usually associated with pituitary adenomas[15] and more rarely with ectopic expression of GH by lung carcinomas[16,17].

Some of the diverse effects of GH are mediated directly by the hormone[18-20]. However, it is generally believed that the major effect of GH is stimulation of somatomedin production in the liver[18,19]. Somatomedins are insulin-like growth factors that promote proliferation of mesodermal tissues such as muscle, cartilage and bone[18,19]. The involvement of somatomedins in the GH response provides an explanation for the growth of animals such as MGH-10, 14 and 16 in which the circulating level of GH was only slightly higher than normal. In these animals, GH produced in the liver may be sufficient to stimulate somatomedin production because the local GH concentration is relatively high. Thus, in these animals, GH may mimic the local paracrine function of some hormones[21]. However, the reason for lack of growth of MGH-3 is unclear.

The implicit possibility is to use this technology to stimulate rapid growth of commercially valuable animals. Benefit would presumably accrue from a shorter production time and possibly from increased efficiency of food utilization. The effects of continuously elevated GH levels on the quality of meat will need to be evaluated. By choosing the appropriate GH gene, milk production may be disproportionately increased considering that GH is homologous to prolactin, that GH of some species binds to prolactin receptors, and that exogenously administered GH has been shown to increase milk yield[22,23]. Having a regulatable promoter may be particularly advantageous for timely expression of GH. Applying these techniques to large animals will be more difficult. Nevertheless, when genes for desired traits can be isolated, this approach should provide a valuable adjunct to traditional breeding methods. Optimizing the conditions for integration and expression of foreign genes in mice should facilitate the eventual application of these techniques to other animals.

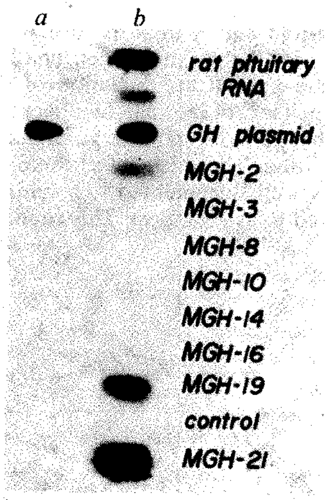

Fig. 4 Slot-blot analysis of liver RNAs from transgenic mice. Mouse liver RNA was purified by homogenizing a liver slice in 1×SET (1% SDS, 5 mM EDTA, 10 mM Tris *p*H 7.5), 200 μg ml^{-1} proteinase K (Boehringer) followed by phenol/chloroform extraction, ethanol precipitation, DNase I treatment (Worthington; DPFF), a second phenol/chloroform extraction, then re-precipitation with ethanol and finally resuspension for A_{260} determination. Liver RNA (30 μg) was diluted to 100 μl in 2X SSC. Samples were brought to 0.5 M NaOH or 0.5 M NaCl and incubated at 65 °C for 30 min. 140 μl of 20× SSC and 160 μl of 12.3 M formaldehyde were added, incubated at 65 °C for another 15 min and applied to a nitrocellulose sheet resoaked in 20× SSC through a slot-blot device[31]. The nitrocellulose was baked at 80 °C for 2 h, prehybridized for 5 h and then hybridized with nick-translated ^{32}P-rGH cDNA[32] (5× 10^6 c.p.m. in 4 ml at 42 °C overnight), washed in 0.1×SSC at 42 °C, and exposed to X-ray film at −70 °C for 24 h. *a*, Samples were treated with 0.5 M NaOH and neutralized with HCl. *b*, Samples were incubated with an equivalent amount of NaCl. 40 and 20 ng of rat pituitary poly(A)$^+$ RNA in the presence of 30 μg of control liver RNA were applied to slots as GH mRNA controls. 1 ng of rGH gene plasmid, also in the presence of 30 μg of control liver RNA, was applied as a base-resistant control.

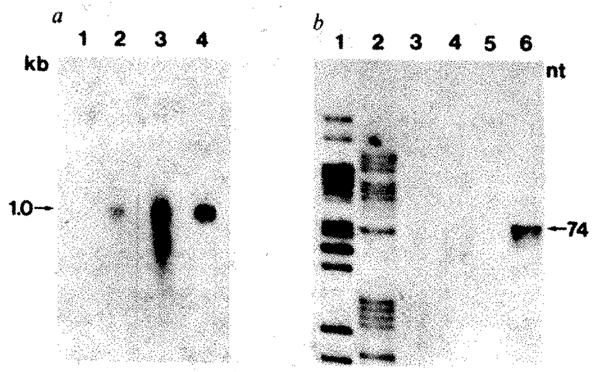

Fig. 5 Structure of MGH mRNAs in liver of transgenic mice. *a*, Northern blot analysis. RNAs were resuspended 10 mM NaH_2PO_4, *p*H 7.4/50% formamide/2.2 M formaldehyde, heated at 65 °C for 5 min, and subjected to electrophoresis on a slab gel composed of 1.5% agarose in 10 mM NaH_2PO_4, *p*H 7.4/0.55 mM EDTA/1.1 M formaldehyde. The running buffer was 10 mM NaH_2PO_4, *p*H 7.4/0.5 M formaldehyde. The gel was stained with acridine orange to identify rRNA markers, photographed, incubated for 90 min with 50 mM NaOH, and then neutralized with two washes of 0.2 M NaOAc, *p*H 4.3. The RNA was then transferred to diazotized paper and prehybridized as described elsewhere[32]. Lane 1, total liver RNA (15 μg) from a control littermate; lane 2, total liver RNA (15 μg) from MGH-21; 3, total mouse pituitary RNA; 4, 40 ng of rat pituitary mRNA, poly(A)$^+$. Lanes 1 and 2 exposed for 48 h; lanes 3 and 4 exposed for 5 h. *b*, Single-strand-specific nuclease protection assay. 20 μg of RNA were hybridized at 47 °C for 5 h in 40 μl of a solution containing 40 mM PIPES *p*H 6.4, 0.4 M NaCl, 80% formamide and 50,000 c.p.m. gel-purified 221-bp *Sst*I–*Xho*I fragment of pMGH end-labelled at the *Xho*I site with ^{32}P (see Fig. 1*c*). The samples were then diluted with 0.3 ml of 280 mM NaCl, 30 mM NaOAc *p*H 4.4, 4.5 mM $ZnSO_4$, 20 μg ml^{-1} salmon sperm DNA and 150 units of mung bean single-strand-specific nuclease (Collaborative Research) and incubated at 47 °C for 1 h. Samples were ethanol-precipitated, resuspended in 90% formamide containing bromophenol blue and Xylene Cyanol FF, loaded on to an 8% acrylamide–urea sequencing gel, electrophoresed for 1.5 h at 2,000 V, dried and autoradiographed for 7 days at −70 °C with an intensifying screen. Lanes 1 and 2, sequencing ladder used for size standards; 3, MGH-3 RNA; 4, control liver RNA; 5, mouse pituitary RNA; 6, MGH-21 RNA.

Another possibility is the use of this technology either to correct or to mimic certain genetic diseases. There are several inbred dwarf strains of mice, one of which, *little*, lacks GH and is about half normal size when homozygous[24]. Thus, introduction of the fusion gene described here or a natural GH gene might restore normal growth to these animals. On the other hand, the experiments described above show that gigantism can be created. Once these mice are inbred to give homozygous stocks, they will provide a valuable model system for biochemical studies on the consequences of excess GH production.

Finally, the exceptionally high levels of GH found in the sera of some of these mice raises the possibility of extending this technology to the production of other important polypeptides in farm animals. The concentrations of GH in MGH-21 serum was 10–100-fold higher than that reported for bacterial or mammalian cell cultures that were genetically engineered for GH production[10,25-27]. This genetic farming concept is comparable to the practice of raising valuable antisera in animals, except that a single injection of a gene into a fertilized egg would substitute for multiple somatic injections; moreover, the expression of that gene is likely to be heritable[9]. This approach may be particularly applicable in those cases where the protein of interest requires special covalent modifications (for example, proteolytic cleavage, glycosylation, or γ-carboxylation) for activity or stability.

Clearly the ability to introduce into mice, and by extrapolation into other animals, functional genes of selected construction offers wide ranging experimental as well as practical opportunities.

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LETTERS TO NATURE

A millisecond pulsar

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The radio properties of 4C21.53 have been an enigma for many years. First, the object displays interplanetary scintillations (IPS) at 81 MHz, indicating structure smaller than 1 arc s, despite its low galactic latitude (−0.3°)[1]. IPS modulation is rare at low latitudes because of interstellar angular broadening. Second, the source has an extremely steep ($\sim\nu^{-2}$) spectrum at decametric wavelengths[2]. This combination of properties suggested that 4C21.53 was either an undetected pulsar or a member of some new class of objects. This puzzle may be resolved by the discovery and related observations of a fast pulsar, 1937+214, with a period of 1.558 ms in the constellation Vulpecula only a few degrees from the direction to the original pulsar, 1919+21. The existence of such a fast pulsar with no evidence either of a new formation event or of present energy losses raises new questions about the origin and evolution of pulsars.

A literature search in 1979 led to the suggestion that the steep-spectrum, IPS source was superposed on a flat-spectrum ($\nu^{-0.1}$) object with a diameter of ~60 arc s located to the west of the 4C position by one interferometer lobe (−31.6 s). Lobe identification errors in the 4C catalogue can occur in regions of confusion such as the galactic plane.

The superposition of two source components, one very compact with a steep spectrum and the other extended with a flat spectrum, was reminiscent of the radio properties of the Crab nebula and its pulsar in the pre-pulsar era[3]. The conjecture was made that the compact component in 4C21.53W was a pulsar as yet undetected due to pulse broadening of its radiation over one period by interstellar scattering. However, the IPS measurement placed an upper limit on interstellar scattering which, in turn, placed a limit on the pulse broadening. The conclusion was that only a very short period pulsar, $P \leq 10$ ms, would have been missed in metre wavelength searches owing to this effect. Searches for such a short period pulsar at centimetre wavelengths, where pulse broadening would be much reduced, were conducted at Arecibo Observatory and at Owens Valley Radio Observatory in 1979 without success.

After the 1979 pulsar searches, Erickson (personal communication) located a steep-spectrum compact source, 4C21.53E, east of the 4C position by one 4C interferometer lobe (+31.6 s). This observation provided evidence against the superposition hypothesis. Furthermore, Very Large Array (VLA) observations at 5 GHz by one of us (D.C.B.) showed that 4C21.53E was a compact double source with separation of 0.8 arc s.

Interest in the extended western object, 4C21.53W, returned when decametric observations at the Clark Lake Radio Observatory showed that both 4C21.53E and 4C21.53W had very steep spectra below 100 MHz (ref. 4). In addition, the Clark Lake observations indicated that the western source showed IPS at 34 MHz. The inferred brightness temperature exceeded $>10^{12}$ K.

Observations of 4C21.53W with the Westerbork Synthesis Radio Telescope (WSRT) at 609 MHz in January 1982

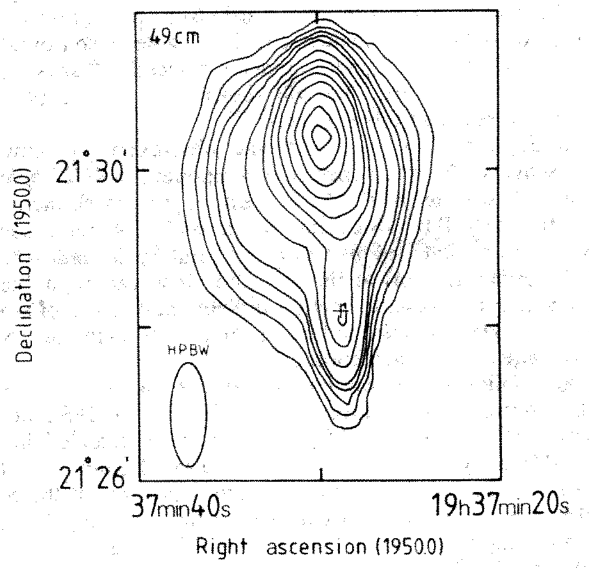

Fig. 1 Image of extended (north) and compact (south, +) components of 4C21.53W from a 12-h synthesis with the WSRT at 608.5 MHz on 15 January 1982. The synthesized beamwidth shown at the lower left is 31.3 × 80.4 arc s in RA and Dec, respectively.

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Table 1 Flux densities of 4C21.53W

Frequency (MHz)	Instrument	Total flux density (Jy)	Compact flux density (Jy)	Compact polarization (%)	Extended flux density (Jy)
430	Arecibo	1.60±0.30	~0.3*	—	~1.3±0.30
609	WSRT	1.15±0.05	0.130	28±2	1.02±0.05
1,380	Arecibo	0.96†±0.05	~0.02*	—	0.94±0.05
1,415	WSRT	0.93±0.02	0.017	15±2	0.91±0.02
2,380	Arecibo	0.90†±0.05	—	—	0.90±0.05
5,000	Bonn[6]	0.91†	—	—	0.92

* Estimated from compact source spectral index between 609 and 1,415 MHz.
† Corrected for beam sizes.

confirmed a suspected position discrepancy based on a Culgoora measurement at 80 MHz (ref. 5) and a Bonn measurement at 5,000 MHz (ref. 6) (Fig. 1). We suspected that the Culgoora position was dominated by the steep-spectrum component and that the Bonn position was dominated by the flat-spectrum, extended component. The division of 4C21.53W into two components, evident in Fig. 1, confirmed our suspicion. The southern and northern components were named 4C21.53W(com) or 1937+214 and 4C21.53W(ext) or 1937+215, respectively. A brief observation at the VLA confirmed the position and steep spectrum of 1937+214. A map of 4C21.53W at 1,415 MHz from a 12-h observation with the WSRT in August 1982 clearly resolved the compact and extended source component (Fig. 2). Recent WSRT observations and 1979 total power observations from Arecibo are summarised in Table 1. The spectra are decomposed into compact and extended object contributions. The Bonn measurement[6] is included for completeness. Erickson's decametric observations of this source are reported elsewhere[7].

The spectrum of the extended source is approximately that of an H II region, $\nu^{-0.1}$. The H 166α recombination line was detected at the Arecibo Observatory at the position of the extended source in November 1982. The line temperature is ~1% of the continuum temperature. The velocity of the line is $+2\ \mathrm{km\ s^{-1}}$ and its FWHM is $\sim 25\ \mathrm{km\ s^{-1}}$. These properties are comparable with those of an ordinary H II region. The small velocity does not distinguish between kinematic distances of 0 and 8.5 kpc.

The proximity of these two components of 4C21.53W on the sky suggests a possible physical connection. The morphology of the 21-cm continuum maps indicates that the exciting star may be displaced 43 arc s northwards from the centre of the H II region defined by the near-circular, low-level contours. On the other hand, the compact source is displaced 117 arc s southward from the centre. We will return later to the possible connection between these objects.

Interest in further pulsar searches was rekindled following the discovery of a steep-spectrum component in 4C21.53W at decametric wavelengths[4]. Momentum for the search increased when the 609-MHz WSRT map was available. A pulsar search sensitive to periods >4 ms was carried out by Boriakoss (personal communication) at the Arecibo Observatory in March 1982 without success. Detection in September 1982 of strong linear polarization at the compact source position in the WSRT maps made a pulsar detection a near certainty.

A new pulsar search was conducted with the 305-m antenna at the Arecibo Observatory on 25 September 1982 at the position of the compact 15 mJy component detected in the 1,400-MHz synthesis observations. Two harmonics of a millisecond periodicity, 1.558 ms, were discovered at the compact source position. The signal was present for only 3 min of a 7 min sample, and was not seen on a following day at either 1,400 or 2,380 MHz.

In November 1982 the pulsar search was intensified at Arecibo. In addition, we planned a search for interstellar scintillation (ISS) based on the possibility that the compact object was small, but not pulsing. Deep ISS modulation was detected at 1,400 MHz at the position of the compact source (Fig. 3). The amplitude was consistent with the flux density found in synthesis observations. The frequency and time correlation lengths, roughly 2 MHz and 5 min, respectively suggested a relatively small dispersion measure to the object, <100 electrons $\mathrm{pc\ cm^{-3}}$. This observation indicated immediately that the compact source 4C21.53W(com) or 1937+214, was extremely small as only pulsars have shown ISS previously. In addition, the modulation bandwidth and time scale were consistent with the single detection in September. On the following day the millisecond pulsar was confirmed.

The waveform of the pulsar contains a main pulse and an interpulse of comparable intensity separated by ~180° (Fig. 4). This morphology repeats precisely the main pulse/interpulse morphology of the Crab pulsar. The Crab pulsar has an additional 'precursor' component preceding its main pulse at metre wavelengths. The full widths at half intensity of the pulse components in Fig. 4 are <125 μs, or 8% of the period. The pulses are readily detected with the Arecibo telescope at 1,400 and 430 MHz with a fast signal averager and integrations of a few hundred pulses when positioned on a peak of the ISS modulation pattern (Fig. 3). The waveforms at both frequencies are similar.

The first observations have resulted in the following parameters: RA (1950.0) 19h 37 min 28.72 s, Dec (1950.0) 21° 28′ 01.3″, Barycentric period 0.001 557 807 (JD 244 5282), Dispersion measure 75 electrons $\mathrm{pc\ cm^{-3}}$. The accuracy of all values is a few parts in the last decimal place. The distance is estimated as 2,500 pc using an average n_e of $0.03\ \mathrm{cm^{-3}}$. There is no evidence for binary motion in timing measurements during the second week in November. Timing observations have been

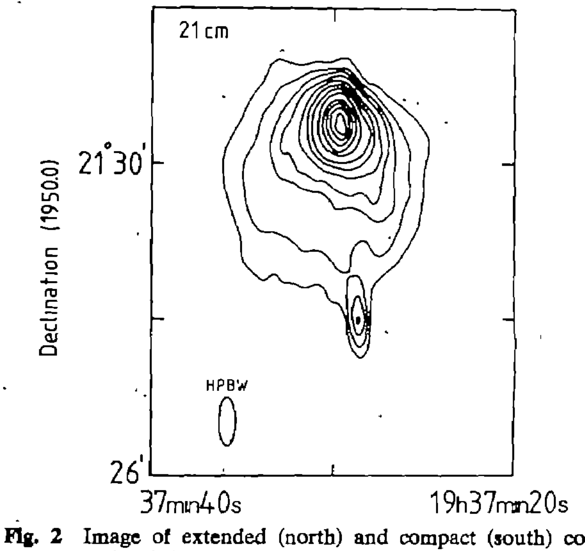

Fig. 2 Image of extended (north) and compact (south) components of 4C21.53W from a 12-h synthesis observation with the WSRT at 1,415 MHz on 8 August 1982. The synthesized beamwidth shown at the lower left is 13.3×37.0 in RA and Dec respectively.

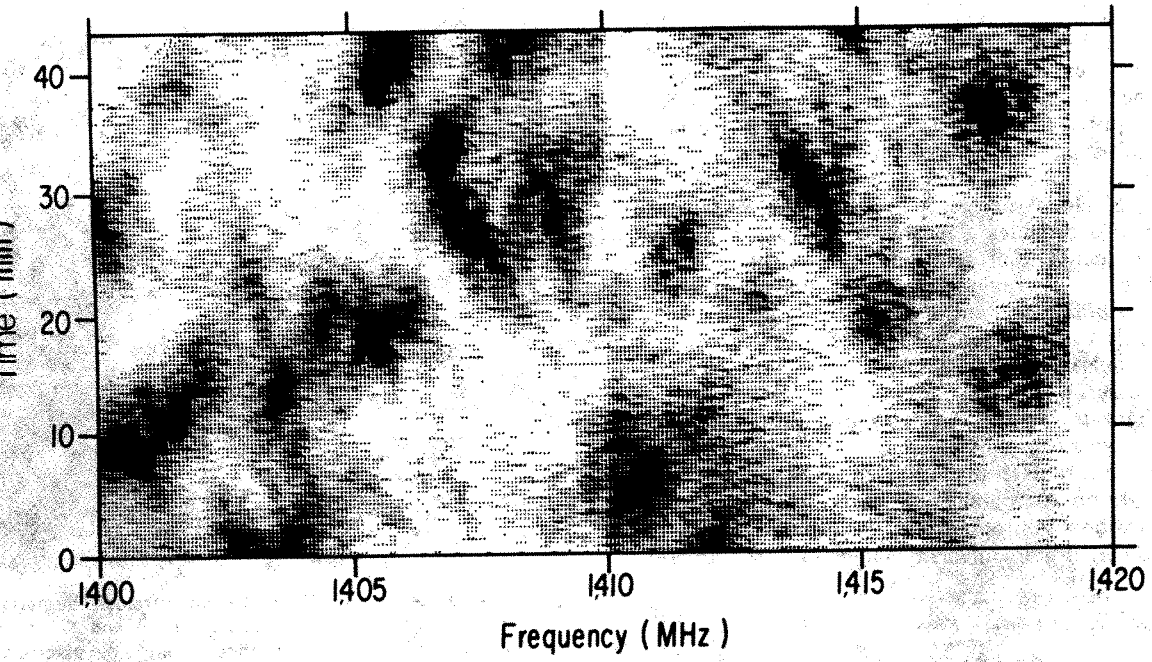

Fig. 3 Interstellar scintillation observation of compact component in 4C21.53W from Arecibo observations on 6 November 1982. Individual spectra were obtained for two polarizations in two 10-MHz bands every 30 s using a 252-channel, 1-bit autocorrelator. Spectra from the two polarization channels were summed and smoothed to a resolution of 100 kHz. The peak intensity in this dynamic spectrum is ~50 mJy.

initiated to determine the period derivative at the Arecibo Observatory. A previous estimate of the period derivative[8], based on a comparison of measured periods in September and November, has not been substantiated by timing data in November. We suspect that the September measurement was corrupted by sampling errors. Analysis of the November observations results in an upper limit to $\dot{P}$ of 10^{-15} s per s.

Our original hypothesis that 4C21.53W was a fast pulsar superposed on an extended synchrotron-emitting nebula was only half correct. Now we are faced with a more profound enigma: the existence of a pulsar rotating near the maximum rate possible for a neutron star with a surprising lack of evidence of energetic activity in the vicinity of the pulsar.

The present rotation rate, 642 Hz, is very near the maximum rate of 2,000 Hz where centrifugal forces balance gravitational forces at the surface of a 1 $M_\odot$ neutron star with 10 km radius. Matter on the equator would have a velocity of 0.13c. Changes in the equilibrium figure of the pulsar resulting from energy losses to magnetic dipole and gravitational quadrupole radiation (discussed below) may occur as abrupt starquakes if there is such a close balance between gravitational and centrifugal forces. The balance could be tipped in favour of gravity if the pulsar is denser than $\Omega^2/G \sim 2\times10^{14}$ g cm^3. The amplitude and frequency of starquakes, observable in pulse arrival time measurements, may be able to distinguish between standard (1 $M_\odot$) and high density models for the millisecond pulsar.

The present rotational energy content is 7×10^{51} erg for a neutron star moment of inertia 10^{45} g cm^2. This energy is comparable to the entire mechanical energy output of a supernova event. A higher density star, as suggested above, could reduce the present energy content if its moment of inertia were smaller than 10^{45} g cm^2. Both the rapid spin and the large energy content are indicative of a young object as energy losses to magnetic dipole and gravitational quadrupole radiation are strong functions of the rotation rate, Ω^4 and Ω^6, respectively[9]. The minimum energy loss for this pulsar will be the observed radio emission which amounts to 3×10^{30} erg s^{-1} assuming a beam solid angle of 1 sr.

The age of this pulsar is puzzling. The maximum spin rate for a neutron star is ~2,000 Hz. A model for the Crab pulsar by Ostriker and Gunn[9] predicts a period decay from this rate to 100 Hz in 1 yr due to gravitational quadrupole radiation. We do not observe such a rapid decay. Furthermore, there is a surprising absence of evidence of any debris from a recent neutron star formation event. Our radio maps show no synchrotron-emitting nebula in the vicinity of the pulsar. Einstein observations place a limit of 1.5×10^6 K for the surface temperature of a neutron star at the pulsar position for the indicated distance of 2 kpc, and exclude the possibility of a synchrotron nebula (D. J. Helfand, personal communication). These X-ray limits do not allow for possible heavy extinction. There is no source at the pulsar position in the COS B catalogue[10]. Lick Observatory observations reveal a 20 mag optical star at the position of the pulsar[11]. In direct analogy to the Crab pulsar, we expect this object will show optical pulsations.

We conclude that despite the large spin and rotational energy, this pulsar is not young. Evidently it has found a way to preserve a large fraction of its orignal energy. Minimal energy loss requires low values for the perpendicular magnetic dipole moment and the gravitational quadrupole moment. The first binary pulsar, 1913+16, is an example of a rapidly rotating neutron star (17 Hz) with a very low moments based on period derivative measurements. Observations of the spin decay will determine the dominant energy loss mechanism.

Two factors lead us to suggest that the pulsar and the H II region are related: (1) The two objects are in the same area of sky in a region of relatively low radio confusion. (2) The brighterst part of the H II region and the pulsar are displaced to opposite sides of the near circular, low-level contours of the H II region.

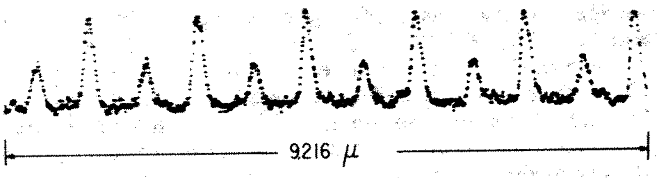

Fig. 4 Waveform of the millisecond pulsar from a signal averager oscilloscope display. Sample spacing is 9 μs. The full trace is roughly six periods, 9,216 μs. The integration lasted ~75 s. The waveform consists of a main pulse and an interpulse separated by nearly 180° of rotational phase. Errors in timing the signal averager and a 20 μs RC time constant are responsible for most of the pulse width.

We propose that the pulsar and the exciting star of the H II region were formerly members of a binary system. One of the components evolved and went through a quiet neutron-star formation stage. Formation of neutron stars in binary systems is also required to explain X-ray and radio pulsar binaries. A large and asymmetric energy and momentum transfer to the neutron star from the other component must have provided the escape velocity necessary to disrupt the binary orbit. This transfer also provides a means for creating a massive, high density object. The present separation of the pulsar from the centre for the H II region suggests an epoch for the disruption event of 7,800 yr ago and a distance of 2 kpc if we assume a typical pulsar transverse velocity of 150 km s^{-1}. Proper motion of the pulsar would be southward in declination of 0.015 arc s yr^{-1}.

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Optical identification of the millisecond pulsar 1937+214

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Backer *et al.*[1] have reported the discovery of a pulsar with the period of 1.558 ms, identified with the source 1937+214 (=4C21.53). The extreme rotational velocity and high energy loss rate of this object make it a very interesting astrophysical phenomenum not only as an important clue for the physics of neutron stars and supernova remnants, but also as a potential strong emitter of gravitational radiation (S. White and J. Arons, personal communication). I present here an optical identification candidate for the millisecond pulsar: it is a 20th mag red object, undetectable on the Palomar Sky Survey prints. Finding charts and offsets from nearby stars are given.

The data were obtained on the night of 14 October 1982 UT, on the Lick Observatory 1-m telescope, in conditions of good seeing (FWHM ≅ 1 arc s). A red-sensitive TI 500×500 thin CCD was used as a detector, together with a sky-suppression red filter, purchased earlier by H. Spinrad. This wide red bandpass has an effective wavelength at about 6,500 Å and the width of ~1,400 Å. Three CCD exposures, one of 300 s and two of 600 s, were obtained. The frames were flatfielded with a 'dome flat', filtered to remove cosmic ray events, and had bad columns masked (standard CCD reduction procedure). The three frames were then offset into coincidence, and digitally stacked together, to increase the signal-to-noise ratio.

Part of the resulting image containing the object is shown in Fig. 1. Figure 2 shows a slightly larger region, enhanced by a new, highly nonlinear algorithm, which will be described elsewhere. The candidate object was found on all three CCD frames, although only the stack is shown here. Figure 3 shows a much larger field, as enlarged from a Palomar print.

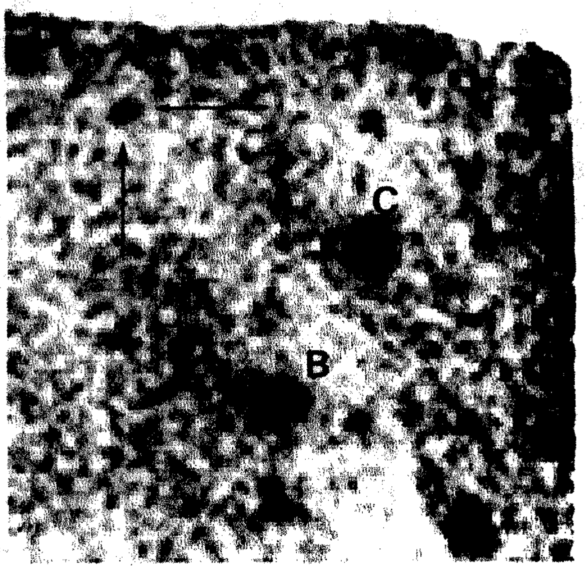

Fig. 1 A section of the CCD stack frame. The pulsar candidate is marked with the arrows. Stars B and C are those from Table 1. The field size is about 50 arc s, the pixel size 0.38 arc s. North is up, east to the left.

I have performed aperture photometry on the digital data of this source. In a pseudo-diaphragm of 6 arc s diameter, I obtain

$$m_r = 20 \pm 1$$

(the error may be an overestimate). The zero-point of the photometry was determined from a standard star, Feige 15, whose exposure was taken immediately after the pulsar field exposures. There is no extended emission associated with this source, down to the limit of these data.

I have also searched for the extended source (H II region ?), detected in radio observations, a few arc min to the north from the pulsar, and apparently associated with it. The result of this attempt was negative, but my data (one 600-s exposure) are of insufficient quality to place any interesting limits on the possible existence of extended optical structure there.

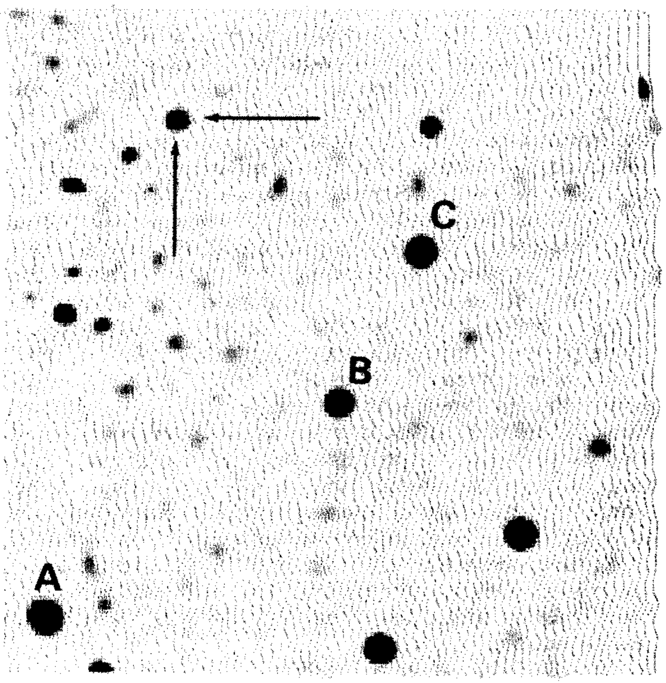

Fig. 2 Similar to Fig. 1, but strongly enhanced with a new, nonlinear technique. The field size is about 1 arc min. Faint 'objects' are not to be seriously believed.

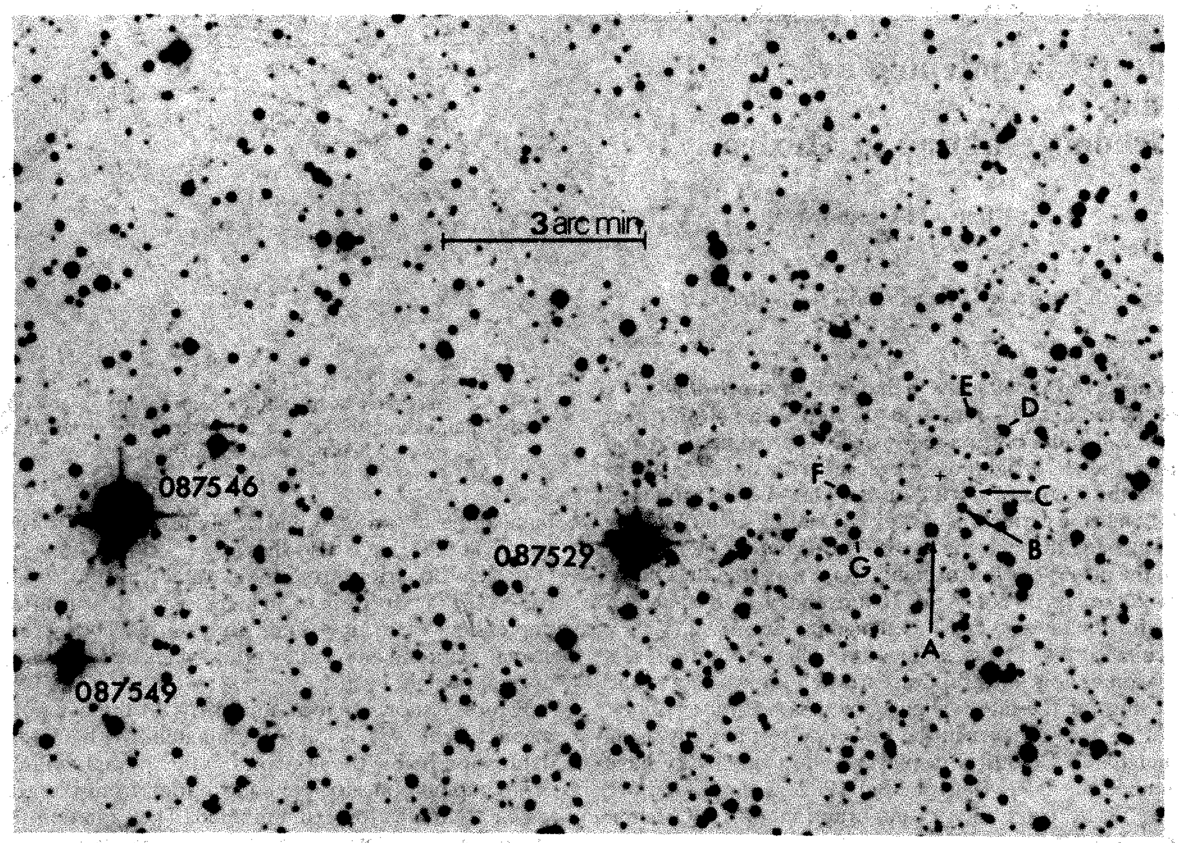

Fig. 3 Enlargement from the POSS chart E-185, showing the pulsar field, and stars denoted as in Table 1. Approximate pulsar position is marked with a cross. Horizontal bar gives the scale. North is up, east to the left. The whole Palomar chart shows high obscuration.

Table 1 lists several stars in this field, as marked in Fig. 3. Positions of the SAO stars were taken from the SAO catalogue. Stars A–G had their positions measured by J. Condon (personal communication), who quotes a random error of ~0.4 arc s. The radio position of the pulsar has been measured by Backer at the Very Large Array.

Pixel coordinates on the CCD stack frame were measured for stars A, B and C, as well as the pulsar candidate, and the faint object south-east from it. The relation between these (CCD) and equatorial coordinates was found, and the position of the optical pulsar candidate was derived from it. I have obtained the following differences:

$$\Delta\alpha \text{ (Condon/radio} - \text{Djorgovski)} = -1.65 \pm \sigma \text{ arc s}$$

$$\Delta\delta \text{ (Condon/radio} - \text{Djorgovski)} = +1.52 \pm \sigma \text{ arc s}$$

where $\sigma \geq 1$ arc s. This error includes Condon's quoted error of 0.4 arc s, and the estimated error of my measurements, which is at least 0.6 arc s. There is also an unknown contribution from distortions of the CCD field (which may add as much as 0.1–0.5 arc s to this error). I believe that the realistic error bar is more like 1.5 arc s. Thus, my optical candidate is within about 1σ error box from the radio position. There is a slight possibility that the very faint object south-east from the candidate star is the correct identification; however, this object may also be spurious (a CCD artefact).

Table 1 Positions and offsets for the stars in the field

Star	α (1950.0)	δ (1950.0)	Star–Pulsar Δα (arc s)	Δδ (arc s)
PSR1937 + 215	19 h 37 min 28.72 s	+21° 28′ 01.3″	0	0
SAO-087529	19 h 37 min 48.85 s	+21° 27′ 06.5″	281.0	−54.8
SAO-087546	19 h 38 min 22.48 s	+21° 27′ 32.9″	750.5	−28.4
SAO-087549	19 h 38 min 25.92 s	+21° 25′ 24.2″	798.7	−157.1
Condon A	19 h 37 min 29.66 s	+21° 27′ 16.9″	13.1	−44.4
Condon B	19 h 37 min 27.69 s	+21° 27′ 36.8″	−14.4	−24.5
Condon C	19 h 37 min 27.12 s	+21° 27′ 50.8″	−22.3	−10.5
Condon D	19 h 37 min 24.90 s	+21° 28′ 45.1″	−53.3	43.8
Condon E	19 h 37 min 27.01 s	+21° 29′ 01.5″	−23.9	60.2
Condon F	19 h 37 min 35.30 s	+21° 27′ 51.3″	91.9	−10.0
Condon G	19 h 37 min 34.60 s	+21° 27′ 14.4″	82.1	−46.9

Further photometry (and, in particular, search for variability) and spectroscopy are needed. A search for optical pulses from this object has been initiated by D. Cudaback and J. Middleditch (personal communication).

Backer *et al.*[1] have determined the dispersion measure towards this source, and from it an estimate for the distance to it, ~2.4 kpc. It has been proposed independently by D. Van Buren (personal communication) that this source may be associated with the Vulpecula OB association, about a degree to the west. The distance to this association is estimated to be ~2 kpc.

This object has almost the same brightness in the present CCD red bandpass, as the faint star just north from the star C, and which is easily detectable on the Palomar red print. However, the object is invisible on the same print. The two bandpasses (CCD and POSS) are not very different, so that colour effects can hardly explain this discrepancy. It is then possible that this object had increased in apparent brightness at least a magnitude over the past couple of decades.

We thank Don Backer for ideas and stimulation, Vicki Lindsay for help in obtaining the data, and many colleagues in Berkeley for stimulating discussions. Lick CCD data taking system and flat fielding program were written by T. Laver and R. Stover. This work was partly supported through a University of California graduate fellowship.

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Twilight IR brightening over India due to El Chichón's eruption in Mexico

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We have observed an unusual behaviour in the twilight sky brightness in the near IR region associated with volcanic dust from the El Chichón eruption in Mexico. Approximately 8 min after sunset the sky brightness increases with time for about 8–10 min in the I band ($\lambda_{\rm eff} = 0.87$ μm). This may be explained by a variation in the extinction of the illuminating solar radiation during its passage through the aerosol layer before being scattered into the observer's line of sight. Here, we estimate a mean height of 22 km, a thickness of 10 km and a drift speed of 19 km h^{-1} for the layer over Ahmedabad in June 1982.

Spectacular visual effects in the form of coloured sunsets following violent volcanic eruptions are well known[1,2], and the time of fading for the late twilight glow has been interpreted in terms of an aerosol layer in the lower stratosphere by using shadow heights[3,4]. Colourful displays in the twilight sky have occurred since the latter half of May 1982 in India, and are thought to result from the recent eruption of El Chichón Volcano[5] in Mexico.

Observations were carried out at Thaltej (23°03′ N, 72°30′ E) near Ahmedabad using a solid state photometer in the I band ($\lambda_{\rm eff} = 0.87$ μm; $\Delta\lambda_{\rm eff} = 0.25$ μm) coupled to a Celestron-14 telescope. At this wavelength (located between two water vapour absorption bands), the troposphere is expected to be more transparent than the volcanic dust-laden lower stratosphere. This wavelength band is therefore ideal for detecting stratospheric dust. Figure 1 shows the time variations of the I band intensity on 8, 9 and 15 June 1982. A normal twilight curve[6] at 0.75 μm is also shown.

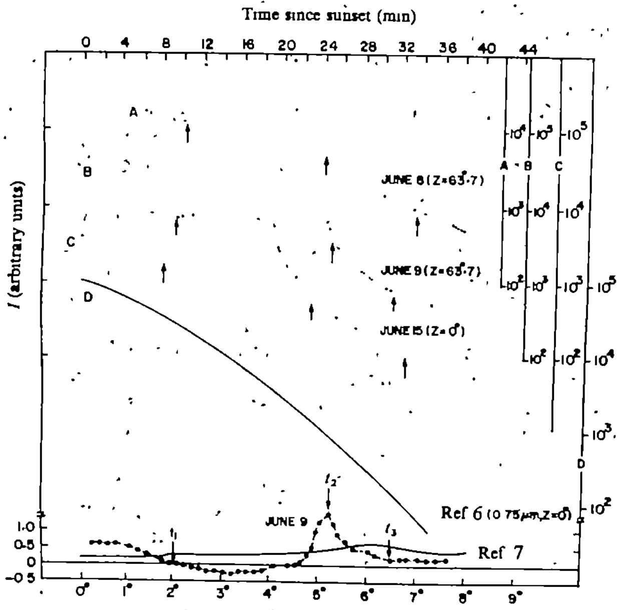

Fig. 1 Light curves A, B, C show the I band intensity (I) variation with time after sunset. D refers to the normal twilight curve at 0.75 μm (ref. 6). The plot at the bottom shows $-d/d\alpha \log I$ as a function of time after sunset for the light curve B corresponding to 9 June 1982. Also shown is the derivative curve for normal twilight at 0.659 μm (ref. 7). Times t_1, t_2 and t_3 decribed in the text have been indicated by means of arrows.

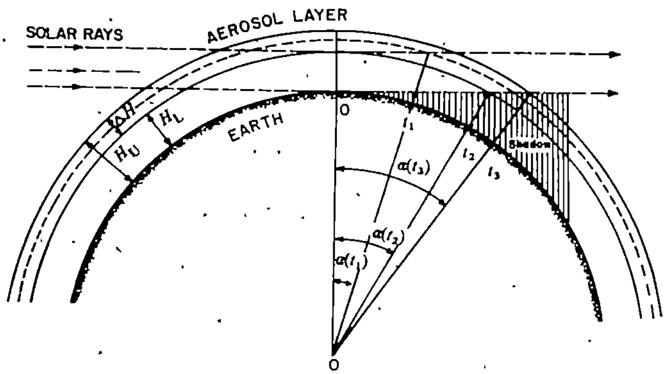

Fig. 2 Geometry of the situation for zenith observations. Solar rays illuminating the aerosol scatterers suffer attenuation during their paassage through the layer.

A clear departure from the normal twilight drop in intensity can be seen in the form of an increase in intensity after a shallow depression occurring ~8 min after sunset. The increase culminates in a broad maximum after which it falls sharply to the normal twilight level.

Figure 2 shows that at sunset ($t = 0$) the aerosol layer at a mean height ($\bar{H}$) is fully illuminated. With increasing solar depression below the horizon (α) the normal twilight intensity drops rapidly, but not the contribution from the aerosol layer which continues to be illuminated. The radiation illuminating the aerosol scatterers suffers increasing extinction with time due to its increasingly long passage through the layer. At time t_1 the central light ray has the longest passage through the layer and the extinction is at a maximum, thereby giving rise to a minimum in the observed intensity.

Due to the curvature of the Earth and the finite thickness of the layer, the total light path through the layer and the extinction are reduced after time t_1. The observed intensity consequently increases, reaches a plateau and then falls gradually due to the increasing attenuation of light reaching the aerosol scatterers by the lower atmosphere. At time t_2 the shadow of the Earth begins cutting off the illumination to the layer from the lower end. At time t_3, the shadow has completely cut off the layer and the intensity drops to the normal twilight level.

A model considering extinction of solar rays in the aerosol layer based on the above geometry has been evolved to explain the observed features. A uniform layer with a thickness of ΔH is considered. The extinction ($A_{\rm I}$) suffered by the light reaching the aerosol is calculated using the relation

$$A_{\rm I}(x,t)=\begin{cases} k_{\rm I}R\left\{\left[\sin^2\alpha(t)+\dfrac{2\Delta H}{R}\left(1-\dfrac{x}{\Delta H}\right)\right]^{1/2} \right. \\ \left. -2\left[\sin^2\alpha(t)-2\dfrac{\Delta H}{R}\left(\dfrac{x}{\Delta H}\right)\right]^{1/2}+\sin\alpha(t)\right\} \\ \quad \text{for } \alpha(t)>\sin^{-1}\left(\dfrac{2x}{R}\right)^{1/2} \\ k_{\rm I}R\left\{\left[\sin^2\alpha(t)+2\dfrac{\Delta H}{R}\left(1-\dfrac{x}{\Delta H}\right)\right]^{1/2}+\sin\alpha(t)\right\} \\ \quad \text{for } \alpha(t)\leqslant\sin^{-1}2x/R\left(\dfrac{2x}{R}\right)^{1/2} \end{cases}$$

where $A_{\rm I}(x,t)$ is the extinction suffered by the I band intensity reaching the observer at time t after scattering by an aerosol particle located at a distance x above the lower height ($H_{\rm L}$) of the layer. $k_{\rm I}$ is the extinction per unit length in the I band, $\alpha(t)$, the solar depression angle at time t, ΔH, the layer thickness and R, the radius of the Earth.

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The model I band intensity at time t is then given by

$$I(t) \propto \int_0^{\Delta H} \exp(-A_I(x,t))\,dx$$

This line of sight integration has been numerically evaluated for various values of k_I and ΔH. Figure 3 shows the result for $\Delta H = 10$ km and $k_I = 3\times10^{-8}$ mag cm^{-1}. The calculated intensity passes through a clear minimum (corresponding to t_1) and then increases to reach a plateau. Despite uncertainties introduced by the unknown normal twilight intensity on which the dust contribution is superimposed, the agreement between the model and the observations in explaining the unusual brightening appears satisfactory.

The times t_1, t_2 and t_3 used in the calculation of layer height and thickness are listed in Table 1. The times have been obtained from $-d \log I/d\alpha$ against t plots. The lower end of Fig. 1 shows a representative plot of this derivative curve for 9 June. A derivative curve for the normal twilight obtained at 0.659 μm is also shown for comparison.

Table 1 Calculation of height and thickness of aerosol layer

Date	t_1		t_2		t_3		ΔH (km)	H_L (km)	H_U (km)
	min	s	min	s	min	s			
8 June 1982	10	20	24	12	33	22	9.4	16.5	31.8
9 June 1982	9	24	25	0	31	12	8 0	17.6	27.5
15 June 1982	8	12	23	0	32	24	9.2	17.0	36.1

The lower height of the aerosol layer (H_L) has been evaluated from time t_2 using the expression[8]:

$$H_L = R\{1-\cos\alpha(t_2)\}\frac{\cos Z}{\cos\psi},$$

where $\alpha(t_2)$ is the solar depression angle at t_2 corrected for refraction due to a double passage through the atmosphere, Z is the zenith angle of the observation and $\psi = Z - \alpha(t_2)$. An upper limit to the height (H_U) is determined in a similar way using t_3. The time t_1 is used in evaluating layer thickness (ΔH) from the relation (valid for zenith)

$$1 - \Delta H/2R \simeq \cos\alpha(t_1) \text{ or}$$

$$\Delta H \simeq R\alpha^2(t_1)$$

For zenith angles departing from zero appropriate corrections to t_1 and hence $\alpha(t_1)$ have to be made before evaluating ΔH.

The analysis yields a mean lower height of the aerosol layer $\bar{H}_L = 17.0 \pm 0.6$ km, an upper limit to the height of the layer $\bar{H}_U = 32 \pm 4$ km, and layer thickness $\overline{\Delta H} \simeq 9$ km. Note that the normal summer tropopause height at 23° latitude is ~17 km. The observations show the unusual brightening in I (0.87 μm) and R (0.66 μm) but only an inflection in the V(0.54 μm) light curve. A spectral index $\beta(I_{scatt} \propto 1/\lambda^{\beta})$ evaluated from the observations and corrected for reddening due to the lower atmosphere yields a value of 3.7 at 15 min before sunset, 1.3 at the time of the peak I band intensity and 3.4 after the passage of shadow through the layer. The low value of β at the time of the peak I band intensity indicates that the radiation received at that time comes predominantly from scattering by the aerosol layer.

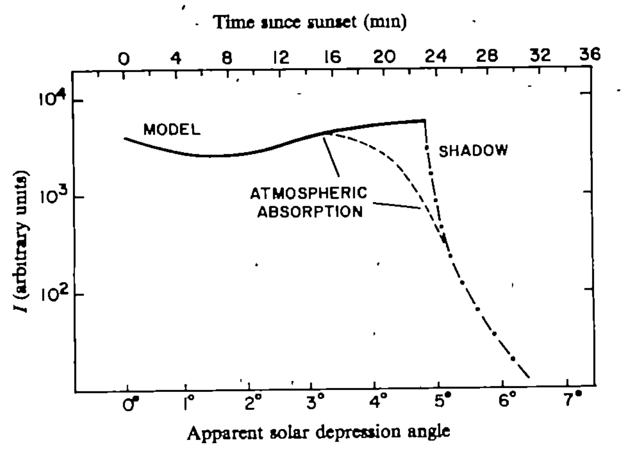

Fig. 3 A light curve computed from the aerosol layer model ($\bar{H} = 22$ km, $\Delta H = 10$ km, $k_I = 3\times10^{-8}$ mag cm^{-1}). The effects of the atmospheric absorption (-----) and the Earth's shadow (—·—·) are shown.

A lower limit to the mass of the aerosol layer M_d can be estimated by using extinction A_I calculated by comparing the peak intensity to the intensity at time t_1. A lower limit to the value of extinction at the time of the maximum length light passage through the layer (~500 km for $\bar{H}$ ~ 22 km) is given by $A_I > 0.763$ mag. This yields

$$M_d > 3.68\times10^{12}(f/Q)\left(\frac{a}{0.15\ \mu\text{m}}\right)\left(\frac{\rho_g}{1\text{ g cm}^{-3}}\right)A_I\,g$$

where a is the aerosol particle size in micrometres, ρ_g the grain density in g cm^{-3}, Q the dimensionless extinction efficiency factor and f the fractional area of the Earth covered by the layer. For a latitude spread of the layer of ±20° f ~ 0.3. Taking a ~ 0.15 μm, ρ_g ~ 2, Q ~ 0.5 we get $M_d \gtrsim 4\times10^6$ tonnes. The earliest positive sighting of volcanic layer features occurred in Ahmedabad about 40 days after the El Chichón eruption, implying drift speed of ~19 km h^{-1} from this delay.

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Anti-phase domain boundary tubes in Ni_3Al

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The mechanical behaviour of ordered alloys has caused much scientific and engineering interest[1,2]. The engineering interest is due to the fact that many ordered compounds, which have unusually good mechanical properties, are incorporated as dispersed particles in and contribute to the high strengths of some high performance commercial alloys (for example, Ni-base superalloys and certain Al alloys). The scientific emphasis has been directed towards understanding the physical basis of the high strengths of these materials and anomalous behaviour such as the increase in yield strength with temperature. We report here preliminary results based on transmission electron microscopy (TEM) studies of the defect structures in the compound Ni_3Al which is the principal strengthening constituent of nickel-base superalloys used in gas-turbine engines: our observations confirm the existence of a new type of defect, anti-phase domain boundary tubes (APB) which we suspect may be an important contributor to the high strengths of these compounds.

In many nickel-based alloys the high-temperature strength is provided by the γ' precipitate based on Ni_3Al, an ordered phase with the $L1_2$ structure. The flow stress of the γ' phase reaches a peak value at about 700 °C. Several theories have been put forward to explain this anomalous behaviour[3] with

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associated TEM studies[4,5] to characterize the dislocation configurations, but these have not succeeded in explaining all features of this behaviour. Recently, the existence of APB tubes in an Fe–Al alloy with B2 ordered structure has been demonstrated[6,7]. The existence of these tubes had been previously predicted[8] and they have been invoked to explain the high work hardening rates of ordered alloys by providing additional drag forces on jogged dislocations[9,10]. In principle, APB tubes should occur during the deformation of a wide variety of ordered alloys. We therefore expect that the investigation of APB tubes in the γ' phase might provide a better understanding of the unusual deformation behaviour of this phase and give some insight into the strengthening mechanisms of Ni-base superalloys.

The early stages of this work have been carried out on polycrystalline specimens of Ni_3Al with columnar grains along the ingot axes. Ingots were prepared by melting the constituent elements and casting into 12.5 mm diameter copper moulds in a vacuum of $\sim 3\times10^{-5}$ torr. The ingots were remelted and directionally solidified in argon atmosphere at 10 mm h^{-1} in a temperature gradient of 15 K mm^{-1} followed by homogenization at 1,200 °C for 48 h.

The ingots were spark cut into rods with diameters ~3 mm and lengths ~6 mm. The rods had the columnar grains oriented along their axes. These specimens were compressed 3–5% at one of the following temperatures: room temperature, 200 and 400 °C. Thin foils for TEM work were prepared from slices of the deformed rods; the final electropolish was carried out in a solution of 5% perchloric acid in ethyl alcohol at −50 °C and 15 V. The foils were examined in a JEOL JEM 100B microscope fitted with a top entry stage which allows ±30° of tilt. The 'weak beam' technique with superlattice and fundamental reflections was used.

The following results were obtained from a specimen with surface close to the (112) plane. The sample was compressed at 400 °C to $\delta = 4.1\%$. Figure 1 shows weak beam images of the same area using three non-coplanar fundamental reflections. Contrast experiments have proved that the stacking faults in these pictures are $\langle 112\rangle$ type. Figure 2A shows dotted lines parallel to [011] or [101] in this area in a semi-weak beam image when the superlattice reflection $02\bar{1}$ was used; the deviation parameter w was ~21 (~2 g was satisfied). These features are not apparent in the fundamental reflection modes (Fig. 1). Figure 2B is a superlattice reflection weak beam image of the same area. The reflection used was $20\bar{1}$. The deviation parameter here is $w\sim42$ (~3g was satisfied).

The lines in Fig. 2B are longer than the corresponding lines in Fig. 2A due to different tilts of the specimen. From the position of Kikuchi lines relative to the diffraction spots, it was possible to determine (1) the sense of the tilting and (2) from the observed length changes, that these lines are along (011) and not [101].

The contrast shown by the lines can be summarized as follows: (1) The defects are along $\langle 011\rangle$, the Burgers vector direction in the material. (2) the defects are visible only when superlattice reflections are excited. (3) The oscillatory contrast in Fig. 2A and B shows that the defects are line defects. (4) The fringes are symmetrical about the centre of the specimen and no fringes occur when $w=0$; this latter effect is characteristic of the contrast from two overlapping APBs[6]. (5) The width of these defects does not change with deviation parameter.

All of these characteristics are as expected for APB tubes. It can be established unambiguously that these defects are not dislocations as they are invisible in any of the three non-coplanar fundamental reflection pictures (Fig. 1) which should image all possible dislocations. Again, the features can not be Lomer–Cottrell (LC) locks since although in f.c.c. structures these are along $\langle 011\rangle$ directions (those with $\mathbf{b}=a/2\,[01\bar{1}]$ are along [011]) they should be visible in Fig. 1A and C, since in these cases the invisibility criterion $\mathbf{g}\cdot\mathbf{b}=0$ does not hold. In fact LC locks are edge dislocations and they should also be visible in Fig. 1B, because the $\mathbf{g}\cdot\mathbf{b}\times\mathbf{u}=0$ criterion does not hold, even though $\mathbf{g}\cdot\mathbf{b}=0$ in that case. Neither can the defects be normal anti-phase domain boundaries. If they were two

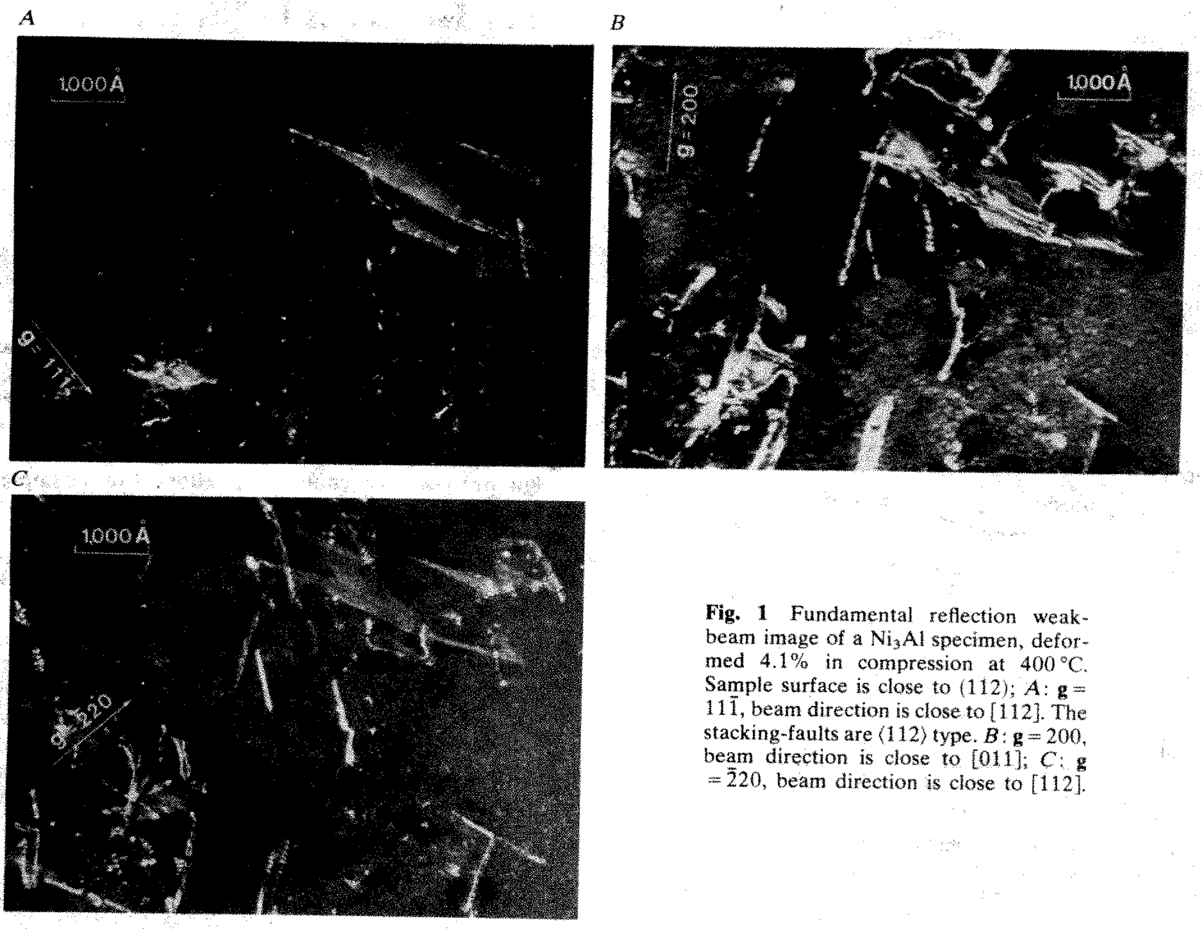

Fig. 1 Fundamental reflection weak-beam image of a Ni_3Al specimen, deformed 4.1% in compression at 400 °C. Sample surface is close to (112); A: $\mathbf{g}=11\bar{1}$, beam direction is close to [112]. The stacking-faults are $\langle 112\rangle$ type. B: $\mathbf{g}=200$, beam direction is close to [011]; C: $\mathbf{g}=\bar{2}20$, beam direction is close to [112].

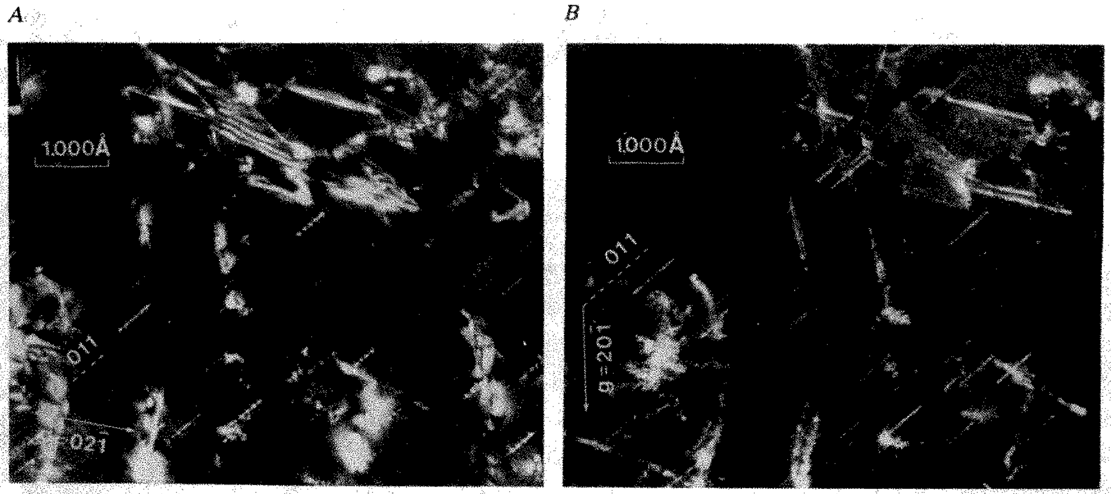

Fig. 2 Superlattice reflection weak-beam image of the same area as that in Fig. 1*A*: $\mathbf{g}=02\bar{1}$, $w \simeq 21$; beam direction is close to [548]. *B*: $\mathbf{g}=20\bar{1}$, $w \simeq 42$, beam direction is close to [326].

dimensional APBs they should not show depth oscillations as in Fig. 2. If they were narrow strips of APBs, they should be connected to partial dislocations, but the latter are strongly imaged using the fundamental reflections. The above discussion leads to the conclusion that the defects along [011] in Fig. 2 are APB tubes.

APB tubes have now been found in deformed ordered alloys with B2 (refs 6, 7) and $L1_2$ superlattices, suggesting that they may be a general feature in deformed ordered alloys. On the other hand their importance in affecting mechanical properties remains to be assessed. Experiments are under way to determine *inter alia* whether the peak in the curve of flow stress as a function of temperature may be associated with the temperature at which climb helps jogs in edge dislocation to align, and in screw dislocations to move non-conservatively, and how APB tubes play a role in superalloys with large proportions of γ' phase.

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A lightning-associated phenomenon and related geomagnetic measurements

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Many observations of phenomena associated with lightning have been made at a considerable distance. Apart from the element of personal danger from direct strike, wind and rain make field conditions unfavourable for observing, and the phenomena are usually very transient. We report here a phenomenon associated with lightning which was observed indoors and at close range following a lightning strike. Magnetic field measurements in the room where the phenomenon was seen are also reported.

In the late evening of 8 September 1981 a thunderstorm approached Te Ngaere (35° 01′ 24″S, 173° 52′ 10″E), 300 km north of Auckland, New Zealand. The one-storey house at Te Ngaere, where the observation was made by Mrs E. V. Sale, is one of a row built behind a sandy beach and occupies the highest point of a low sand-bar at the mouth of a swampy valley. The house is constructed of timber with a cement-tiled roof and steel-reinforced concrete floor. The living-room has a partition (behind the piano in Fig. 1) forming an entry way about 2 m wide. There is a French door with a corded woollen mat just inside. The floor is covered with felt-lined linoleum. Mrs Sale had brought in some wet steel tools, which she had roughly dried and laid out to dry fully on a doubled sheet of newspaper 60 cm by 40 cm (Fig. 1). She was kneeling at the east side of the paper bending over the tools when another heavy lightning strike occurred, just outside the house. Mrs Sale is an amateur astronomer and impressed us as a reliable observer. She related the events as follows:

> The next lightning seemed directly overhead and very bright and was accompanied by a simultaneous very loud clap of thunder. I looked up as the whole house shook and then looked down and saw a flow of light come in under the door. It settled in a blob near the edge of the area where the tools were laid out. It was not in any true shape but about 3 or 4 inches long and 2 inches wide, moving along the floor, less than half an inch thick, seemingly fluid in shape and texture. It reminded me of quicksilver, being a bluish-silver colour and it had rounded sides like a blob of mercury. It was brighter at the edges than in the middle, but it did not seem, especially in the light of the room, to glow, nor did it give out sparks.
>
> From the central body arms flowed out like runs of oil among the tools. The trails weaved through the tools—not actually over them but round them—moving back into the main body of the blob and then going out doing the same kind of movement over again. There was no sound or smell. The arms finally all went back into the blob which disappeared again suddenly out under the door. There was no bang and when I ventured to touch the tools there was no charge on them.

We visited the house on 16–18 November 1981. From this visit together with subsequent correspondence, a more detailed account can be given.

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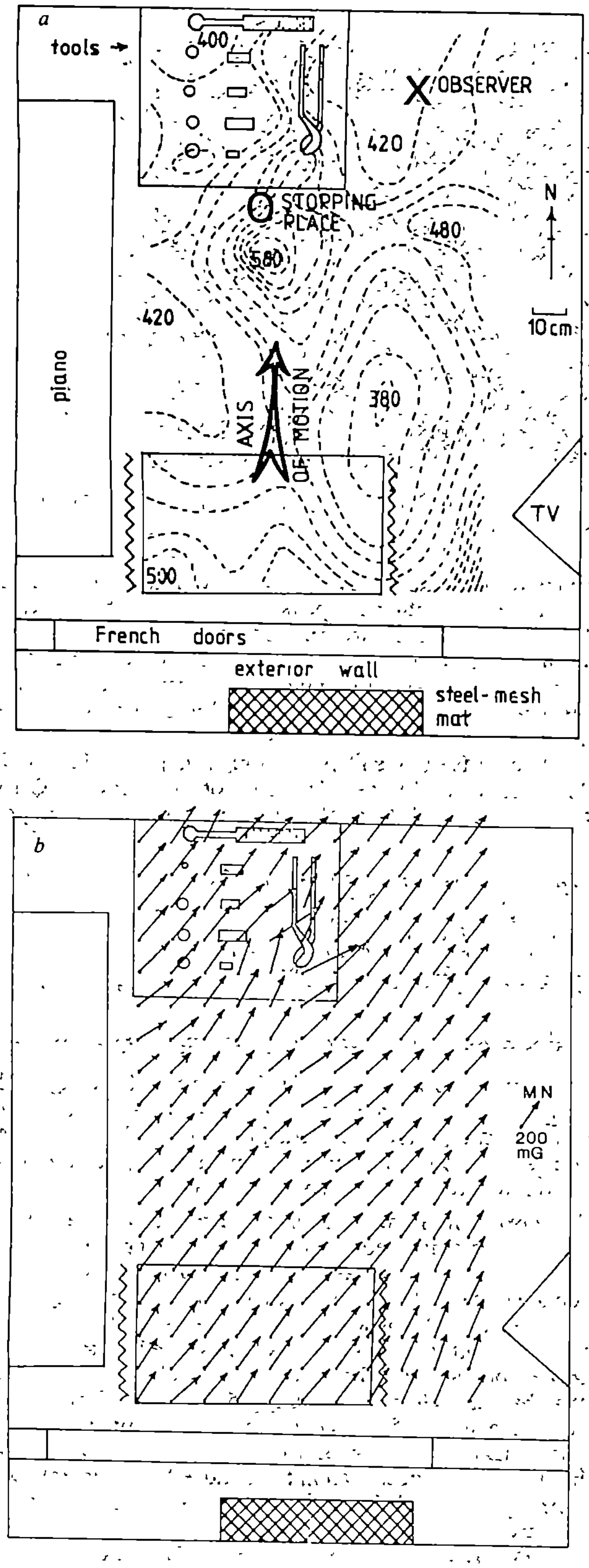

Fig. 1 *a*, Sketch of the region in which the body was observed, showing the observer's position and the path of the body. The vertical magnetic field intensity in milligauss is superimposed, with a contour interval of 20 mG. *b*, Horizontal component of magnetic field, over the same area. Intensity scale is given on the right.

The body was opaque, and must have looked like a faintly luminous blob of milky glass. No trace of it was left anywhere. A strike point could not be found for the lightning and there was no sign of damage to the exterior television antenna, vertically above the set in Fig. 1, or to a large pohutukawa tree a few metres south-east of it. The door was found to have a tapering vertical gap near the bottom, close to the floor about 10 cm long and 0.5 cm wide.

The lighting in the area of the living-room was dim and indirect, coming mainly from a shaded 60 W standard lamp about 4 m away. In an experiment Mrs Sale felt that a blob of freshly poured lead replicated the luminosity of the object when it was illuminated by a candle 10 cm away. We estimate the power emitted as light therefore as $\sim 2\times10^{-4}$ W; no noticeable heat was emitted. Two timing measurements were done as Mrs Sale rehearsed the motion of the body by pointing. The two agreed, giving 4 s for each travel and 7 s halt, a total of 15 s.

As the phenomenon occurred during a thunderstorm and immediately after a heavy strike of lightning in the vicinity, it may be related to that broad group of events currently classified as "ball lightning"[1–3].

Magnetic fields in the vicinity were measured with the tools in place on 17 November 1981. This may be of particular interest as although many accounts of ball lightning refer to iron objects such as axes, stoves and tramlines only one previous author[4] has explored magnetic effects. The geomagnetic field at floor level was mapped near the path of the body with a laboratory fluxgate magnetometer (Schoenstedt DM-2220). Three-axis measurements were facilitated by setting the unidirectional probe in a wooden cube with 6.3-cm sides and taking readings with successive orthogonal faces of the cube resting on the floor and squared against a wooden rule. Each of the observed elements varies over the floor by ~20% which is not unusual for steel-reinforced concrete.

The ambient geomagnetic field is steeply dipping at about 60° above horizontal, and the body was evidently constrained to move in the horizontal plane. If the body contained free dipoles such as current loops, then after alignment by the field it would be expected to experience a force towards the region of most intense vertical field, and possibly move in that direction. It will be seen in Fig. 1 that there is a close correspondence between the path of the body and the 'ridge' of most intense vertical magnetic field. These observations are therefore consistent with the body containing free magnetic dipoles of some kind.

Since the path of the body is a little offset from the line of vertical-component maxima, a capricious force such as a current of air is implied, which might also explain the retirement of the body. It seems reasonable to attribute to draughts the major action in moving the body, the initial entrance and wavering shape strongly suggest this. The living-room is large and well ventilated so that significant and very variable draughts might be expected at floor level given the rapidly-changing winds which would accompany a thunderstorm. Mrs Sale plainly regarded the body as being liquid because it had a surface with wavering blob-like outline and must have been denser than air.

No firm conclusions as to the nature of the body seem possible. The close association with lightning suggests that the strike was the prime cause. The body appears to have been denser than air, had a surface like a liquid, although it was non-wetting, and had a milky-blue luminous appearance. Its radiant energy was in the order of 2×10^{-4} W, observed for 15 s. Wind draughts seem to have been the dominant transport mechanism. From mapping the geomagnetic field we found a weak correlation between the motion of the body, and the regions of most intense vertical field, as would be expected if the body contained free magnetic dipoles or current loops.

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Extensive underthrusting of undeformed sediment beneath the accretionary complex of the Lesser Antilles subduction zone

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Along the eastern margin of the Caribbean Plate, which overrides the Atlantic oceanic lithosphere at ~20 km Myr^{-1} in an easterly direction[1], east of the Lesser Antilles island arc, lies a wide accretionary complex (Barbados Ridge Complex); 260-km wide at the latitude of Barbados and >20-km thick beneath Barbados[3,4], it increases in width and thickness from north to south[2] (Fig. 1). The style of deformation varies considerably along the eastern margin of the complex[2,3,5–11], and is related to the thickness and type of sediment on the Atlantic Ocean floor. A common feature is that a proportion of undeformed sediment from the ocean floor is brought beneath the deformed sediments of the accretionary complex, and in some areas a clearly defined seismic reflector separates the deformed and undeformed sediments, which has been interpreted as a decollement surface[5,12,13]. A seismic section from an investigation of the accretionary complex, conducted from RSS *Discovery* in 1980 using 12-channel seismic reflection and long range side-scan sonar (GLORIA), shows undeformed sedimentary rocks carried horizontally 74 km beneath deformed rocks forming the accretionary wedge. We report here that the low shear stresses required on the decollement between the deformed and undeformed rocks are made possible by porewater at pressures close to that of the lithostatic load.

The longest of three seismic profiles run across the eastern margin of the complex 50 km north of DSDP Leg 78A, Site 543 (X–X^1, Fig. 1) shows that a reflector with layered reflectors between it and the rough surface of the oceanic basement below, extends without significant interruption for nearly 45 km from the edge of the complex beneath a westward thickening wedge of material characterized by disturbed discontinuous reflectors (Figs 2 and 3). Beyond a region of disturbance, a reflector can be seen again continuing above the oceanic basement to the end of the section, some 74 km from the edge of the complex. This reflector and its apparent continuation are interpreted as a decollement, and the implication of this is that virtually undeformed sediment is carried on the ocean crust beneath the overlying accretionary complex for at least 45 km, and probably more than 75 km. Similar 'decollement' reflectors have been reported from other forearc accretionary complexes where sediment entering the complex on the ocean floor is of at least moderate thickness[17–19]. The previous greatest landward extent of such a reflector, 32 km from the edge of the accretionary complex[20], was demonstrated at the Nankai Trough off southern Japan. Extensive decollements are also well known in mountain belts on land[21–24].

It was the principal objective of DSDP Leg 78A in 1981 to drill through the accretionary complex near its leading edge, penetrating deformed sediment, the decollement, the undeformed sediment sequence, and finally the oceanic igneous basement[14–15]. Three sites were drilled on the northern flank of an oceanic basement ridge (the Tiburon Rise) where relatively shallow bathymetry and thin sediments provided favourable conditions (Fig. 1). The decollement was successfully reached in Hole 541, but poor drilling conditions prevented penetration of the undeformed sequence underneath.

The presence of a clearly recognizable decollement constrains interpretations of the formation of the accretionary wedge above it, because the thickness of sediment entering is known, and no further sediment is added from beneath, where the decollement is unbroken. This is important for calculations relating volume of the accretionary wedge and uplift of the surface to the rate of sediment input[25]. Currently, only the upper third of the sedimentary sequence on the Atlantic Ocean floor is added to the leading part of the complex, and if the decollement is stratigraphically controlled, a smaller proportion was added in the past.

East of the accretionary wedge, no clear difference can be seen in the character of the seismic section above and below the reflector which becomes the decollement, but drilling at Site 543 showed that it seems to lie at a change from Lower Eocene mud to radiolarian claystone, and also corresponds to an increase in the cohesion of the rocks from 40 to 100 kPa.

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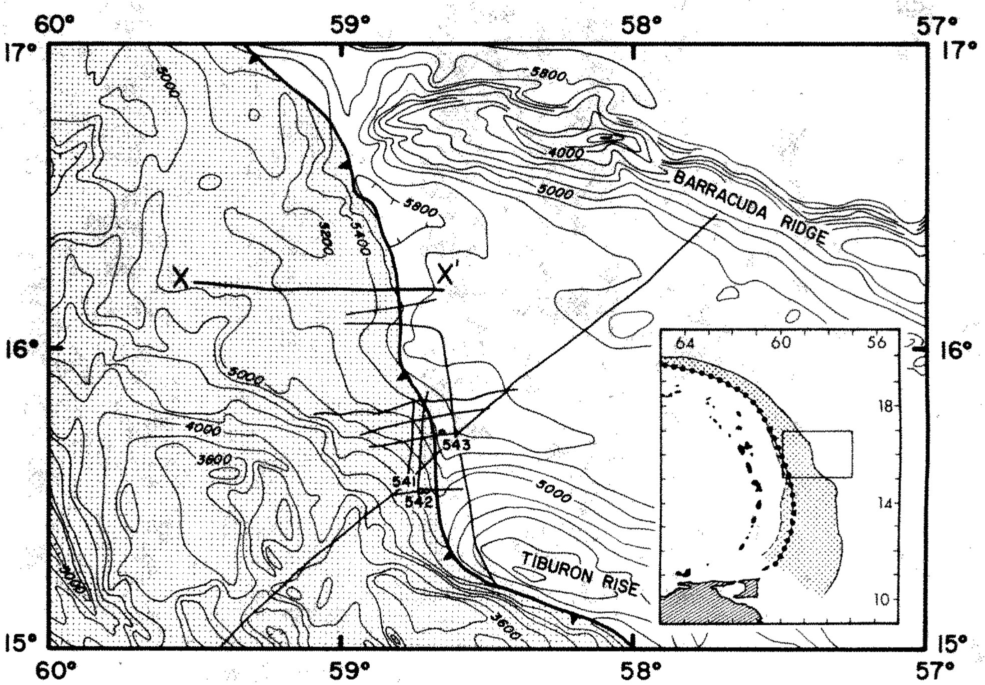

Fig. 1 Map of the front of the accretionary complex of the Lesser Antilles subduction zone, east of Guadeloupe. The area of the complex is stippled, and its front is shown by a heavy line. Also shown are multichannel seismic reflection lines including that shown in Figs 2, 3*a*, *b* (X–X′), and the drill sites from DSDP Leg 78A. Bathymetric contours are at 200-m intervals. The inset shows the area of Fig. 1 in relation to the whole of the accretionary complex (stippled) and the Lesser Antilles island arc (black island shapes). The axis of negative gravity anomaly is shown by dotted line.

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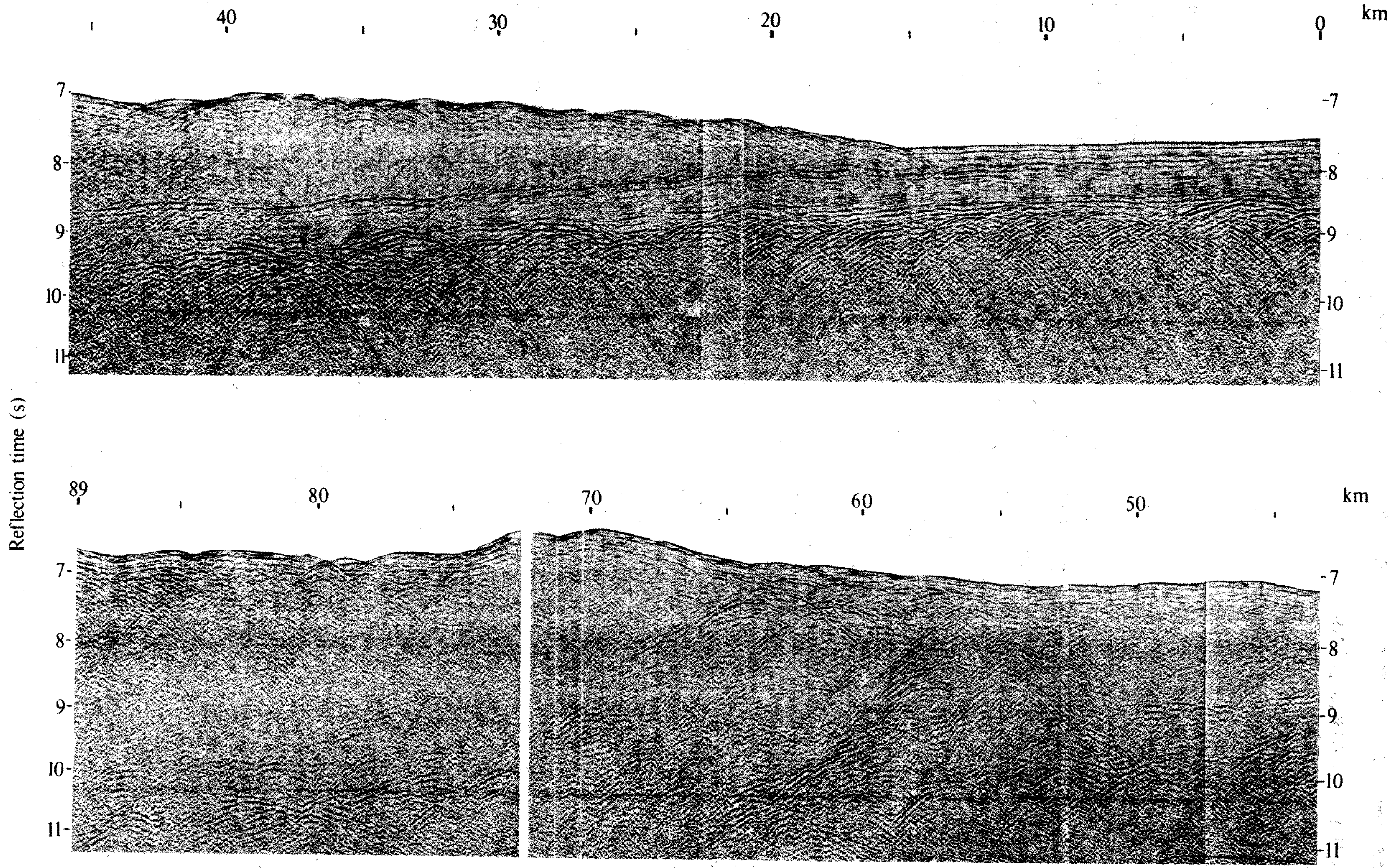

Fig. 2 A 12-fold stacked and deconvolved seismic reflection section across the front of the accretionary complex (X–X′ in Fig. 1). The oceanic basement can be seen beneath the complex, easily recognized from its rough surface which propagates prominent hyperbolae. Above this, another prominent reflector, interpreted as the decollement surface above a sequence of undeformed sediments, can be traced to nearly 60 km from the right-hand end of the section where it becomes obscure, but between 75 and 89 km it can be seen again above the basement, giving a total length of 74 km from the edge of the complex.

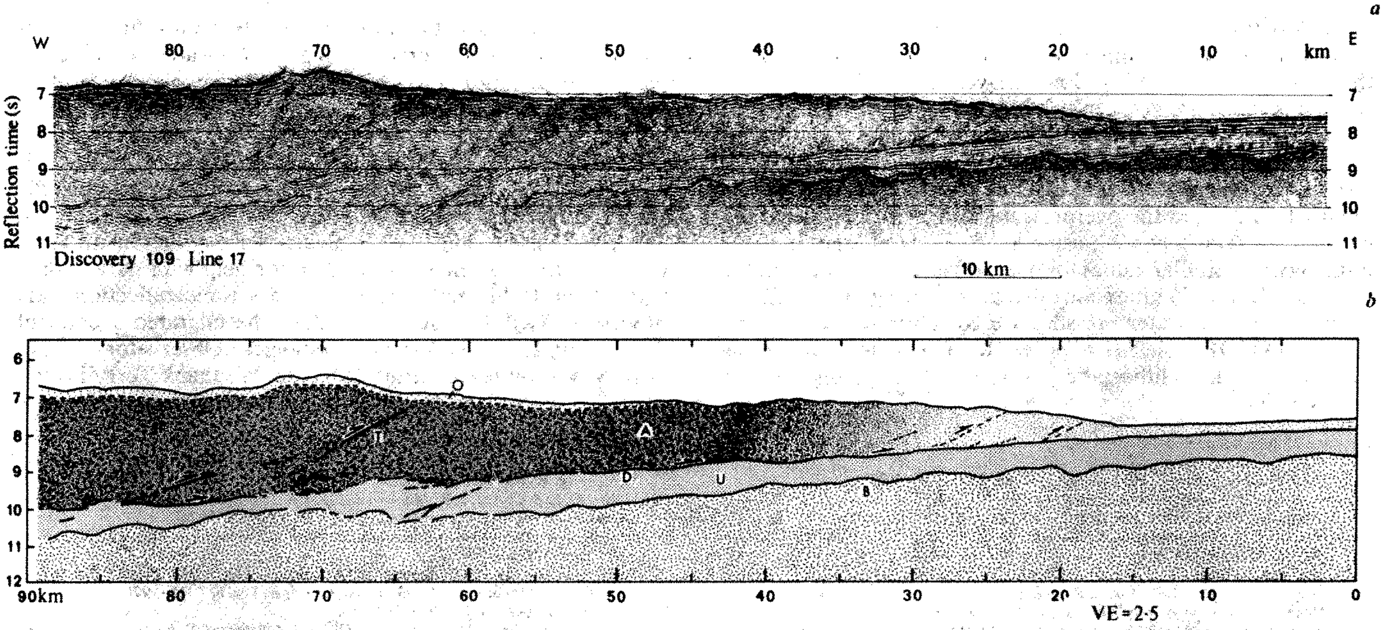

Fig. 3 *a*, Migrated version of Fig. 2. Most, but not all, of the more prominent reflectors have been highlighted. Of particular importance, apart from the basement and decollement, are the two prominent westward dipping reflectors, between 60 and 85 km from the right. They and other shorter dipping reflectors between 15 and 20 km from the right are interpreted as thrusts. *b*, An interpretational cross-section of the accretionary complex, based on Figs 2 and 3*a*. The wedge of accreted and deformed rocks (A) thickens rapidly over the first 25 km, where rapid compaction and movement along closely spaced thrusts, which are more abundant and closely spaced than the few depicted, appear to be the dominant deformational processes. The wedge retains a nearly constant thickness for another 20 km until it increases thickness again by movement along the widely spaced major thrusts (T), which produce uplift of the seabed. Beneath the decollement (D) sediment is undisturbed (U) until it reaches the region of the major thrusts, where some of it may be stripped off into the wedge. The dipping reflectors shown just above the basement (B) between 60 and 65 km are probably also reflections from a basement high slightly out of the plane of the section. Above the complex lies a thin layer of sediment (O) which was deposited directly on to it. The increase in the density of the ornament of the accretionary wedge reflects the inferred increase in density of the rocks, resulting from tectonic compaction. Vertical exaggeration of the relief of the seabed is 3.3 : 1. The average vertical exaggeration of features in the rocks beneath the seabed is 2.5 : 1.

The western end of the section is ~68 km east of the axis of the trough in the basement beneath the accretionary complex, revealed by gravity and seismic refraction data, where it is inferred that the ocean crust passes beneath the crystalline crust of the Caribbean Plate, and so it is not possible to say whether the layer of undeformed sediment is taken deeply into the subduction zone proper or stripped off and added to the base of the accretionary complex.

The rate of thickening of the accretionary wedge is greatest at the leading edge, where the angle of its taper is 5.4°, and decreases arcwards until, between 38 and 54 km from the right of the section there is very little significant increase in thickness with a taper angle of 1° (Fig. 3*b*). There are probably two main mechanisms for the initial thickening: imbricate stacking of small thrust slices, and direct horizontal shortening of the sediments accompanied by the expulsion of porewater during tectonic compaction. Thrusts with a vertical spacing of ~150 m were encountered at Sites 541 and 542 of DSDP Leg 78A, and their presence in the section is indicated by poorly defined westward dipping reflectors near the front of the wedge (Figs 2 and 3*a*). The small scale of the structures at the toe of the complex makes them difficult to recognize on the seismic section which, with a trace spacing of 50 m, does not resolve well reflectors shorter than 0.5 km. The initial thickening of the wedge will tend to reduce the deviatoric horizontal stress acting in the wedge, and it is probably accompanied by an increase in the strength of the rocks as they compact, so that ~22 km from the front some balance seems to be achieved with the rocks forming the wedge able to withstand the stress without any large increase in strain. Further significant thickening of the accretionary wedge seems to proceed by movement along much more widely spaced thrusts such as that seen some 50 km from the front of the wedge, but probably with little change in the strain of the rocks within the thrust slices (Fig. 3*b*). The point at which the wedge begins to increase in thickness again is also close to where the decollement first appears to suffer disruption.

There is little direct evidence on the state of compaction of the rocks forming the wedge. The rocks sampled at DSDP Sites 541 and 542 (refs 14, 15) showed a small but significant increase in density of ~13% from their equivalents at the reference Site 543 in the undeformed sequence in front of the complex, with an accompanying decrease in porosity, but no significant change in seismic velocity. An analysis of the stacking velocities used to produce Fig. 2 and the most effective velocities required to produce the migrated section of Fig. 3*a* was unable to determine any significant change in the seismic interval velocity of the rocks forming the wedge with distance from the front, other than what might be expected from the increase in thickness. This approach was of limited accuracy, however, because of the relatively small aperture of the seismic streamer compared with the depth of the reflectors, and so only a very strong lateral change in velocity is ruled out. A study of sonobuoy data in this area[23] also failed to show any strong lateral variation in velocity.

The small angle of taper between the top and bottom surfaces of the accretionary wedge and the low strength of the rocks forming the wedge and the underlying sequence[14,15], indicates that the shear stresses on the decollement must be very low[27–29]. The dynamics of a thrust wedge in which the stresses in the wedge exceed its strength have been analysed by Chapple[27], who derived the following relationship, where the angles of slope of the base and surface are small:

Shear stress at base of wedge
= Density × Gravity × Angle of slope of surface
× Thickness beneath a horizontal surface through the tip of the wedge
+ 2 × Yield stress of wedge × Angle of slope of base.

Applying this formula to the region of the wedge with a taper of 3° between 21 and 38 km, assuming that rocks are saturated with water but not overpressured, and behave as a Moho–Coulomb material with an angle of friction of 30°, the shear stress on the decollement is computed to be 0.44 MPa. This is much less than the 8.5 MPa expected from the load imposed by water saturated sediments and a coefficient of friction on the decollement of 0.85 (following Byerlee's law[30]). To reduce the stress, the porewater in the zone of the decollement must be overpressured[31] to value which is 0.97 of the load which would be imposed by the wedge if no water were present. If clay minerals reduce the coefficient of friction on the decollement[32] to 0.2, then the overpressure would be 0.81 of the dry wedge load. Davis *et al.*[29], using a different formulation, have obtained very similar values for overpressured porewater in the Barbados Ridge Complex and other accretionary wedges. These estimates of porewater pressure are corroborated by the discovery in DSDP Hole 542 of porewater at a pressure very close to that of the lithostatic load in the region of the decollement[14,15].

We conclude that this long decollement only functions as consequence of the overpressuring of porewater contained in the sediments forming the accretionary complex and undeformed sequence beneath it.

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Rainfall over the Arabian Sea during the onset of the 1979 monsoon

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One path to understanding the summer monsoon of south-east Asia lies through its regimes of rain. There the main scales of interest range, on the small side, down at least to component rainstorms: that is, extending over hundreds of kilometres and lasting about a day. On the large side, interest might be extended to include the whole quasi-planetary monsoon system, although here, for practical reasons, the limits are taken to be 1 week and several thousands of kilometres. The main branch of the summer monsoon forms over the Arabian Sea. The rainfall of this sea, roughly 3,000 km in its dimensions, is known to us from about 40 coastal stations, mostly in India, plus six instrumented islands and the reports of seagoing ships. The ships stay mainly in a few lanes and in any case only record the frequency of rain, not the amount. Thus, even taken all together, these reports are hardly adequate for building a picture of the monsoon rains. We report here a new technique for inferring daily rainfall from visible or IR images obtained by a geostationary satellite, applied in a simplified form to the Arabian Sea during the onset of the 1979 summer monsoon. The rain area estimated increased spectacularly (from 1 to 14%) over a 1-week period coincident with the appearance of the Somali jet and development of the onset vortex.

Some scientists have exploited low-orbiting satellite observations of the Arabian Sea: C. S. Ramage, using visible imagery (personal communication), and Kidder and Vonder Haar and Rao *et al.*, using microwave data[1,2]. In each, the scale of the estimates—5 days or more—is too long to be of much use in portraying the monsoon through its development.

The 1979 Monsoon Experiment, which had an Arabian Sea component, may afford the best opportunity yet to build an evolutionary picture of the onset rains. The basis for this claim is the Indian Ocean Geostationary Operational Environmental Satellite (GOES IO). This satellite, positioned over the Equator at 60 °E, returned a visible and/or IR image, of 1–8 km resolution, once in almost every hour of the days from 11 to 21 June, a period which coincides almost perfectly with the onset of the 1979 monsoon.

To map daily rainfall over the Arabian Sea as the 1979 summer monsoon became established, drawing on several studies (see refs 3 and 4), we hypothesize the following: (1) four rainrates (nil, light, moderate and heavy) can be inferred consistently at any hour from a sequence of hourly visible or IR images; (2) the nil-rate class predominates; (3) most nil and heavy cases can be decided solely on the basis of brightness; (4) time change of brightness and pattern are important in the remaining cases; (5) most of the data in a single image are redundant; and (6) visible and IR data are complementary.

One special constraint follows from the enormous volume of data generated by the GOES IO imaging instrument; we cannot look at every element of every picture. In fact, this kind of problem could only be addressed by training a machine to take repeated measurements and make simple decisions.

The approach that has been taken is to imagine a network of gauges, distributed over the basin of the Arabian Sea. For each hour at each gauge a meteorologist, assisted by a computer, infers from satellite pictures whether the rainfall was light, moderate, heavy or nonexistent. The sum over 24 h of these rainrate class assignments yields an estimate $\hat{R}$ of the daily rainfall R. Thus, if $\hat{r}_i$, $i = 0, 1, 2, 3$, is the estimate of hourly rainfall attributed to the ith rainrate class and f_i is the frequency of the ith rainrate class over 24 h,

$$R \approx \hat{R} = f_0\hat{r}_0 + f_1\hat{r}_1 + f_2\hat{r}_2 + f_3\hat{r}_3$$

or, since $\hat{r}_0 \approx 0$

$$\hat{R} = f_1\hat{r}_1 + f_2\hat{r}_2 + f_3\hat{r}_3$$

Each $\hat{r}$ is a constant to be determined by multivariate least-

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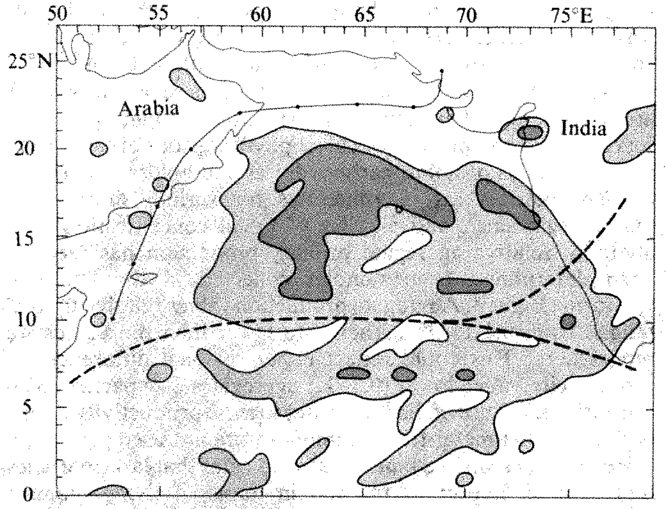

Fig. 1 Rain frequency for 17 June 1979. Stippling marks the grid points where rain was registered at least once during the day. The darker stippling shows where the heavy rain class was recorded at least once. Also shown are the axis of the Somali jet (dashed line) and the centre of the onset vortex (circle, at 16° N, 67° E), both from ref. 7 and the edge of the monsoon inversion (knotted line), from ref. 9.

squares regression of $\hat{r}$ on R, where R is measured by a gauge or radar.

Rain is estimated from a sequence of Earth-located (navigated), registered satellite pictures, each image of the sequence being spaced an hour or so from the others. There may be an IR sequence and a visible sequence or either alone. Images are displayed and analysed on the University of Wisconsin Man-computer Interactive Data Access System, McIDAS[5].

Rain has been estimated by this so-called grid history technique for the area from the Equator to 28° N and from 50° E to 79° E. Initially, the resolution of the grid across this area was 0.5°. It has since been relaxed to 1°. Eight days have been processed, 11–18 June 1979.

Applications of this technique to parts of the South China Sea and the eastern Atlantic Ocean when gauges or radars were present suggest values for the coefficients in the ratio of 1:4:10. So far it has not been possible to acquire enough ground observations of rain around the Arabian Sea to run the regression there. Thus, only rain class frequencies are presented here.

A photographic view of rain frequency for 17 June 1979 is shown in Fig. 1. At this stage of the monsoon, rain was extensive over the Arabian Sea but largely absent over India. A band of mostly light to moderate rain, centred near 7° N, angled north and north-west along the Malabar coast of India, then merged with a large mass of mostly heavy rain in the north-central part of the Arabian Sea. The Somali jet bisected the rain area between the upper and lower masses, and the edge of the rain region lay 200–400 km east and south of the edge of the monsoon inversion.

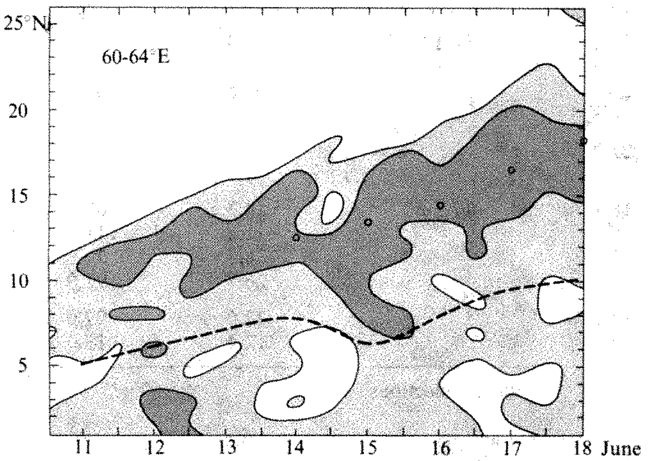

Fig. 2 Time–latitude section of rain frequency averaged over a strip from 60 to 64° E. The convention is the same as for Fig. 1.

Time changes in rain class frequency averaged across a longitudinal strip through the centre of the Arabian Sea (60–64° E) are shown in Fig. 2. Quite consistently, the heavier rain occurred on the north side of the rain area, to the left of the axis of the jet. The rain area spread steadily northwards, at ~150 km day^{-1}, or twice as fast as the northward movement of the jet axis.

It is expected from simple jet stream dynamics[6] that over the Arabian Sea, rain would be heaviest to the right of the axis of the Somali jet. The departure observed here is apparently due to low level convergence occurring with the onset vortex, a cyclone which first appears on 14 June in the 850-mbar analyses of the atlas of Krishnamurti *et al.*[7] The possibility has been suggested[8] that the onset vortex received kinetic energy through baroclinic processes (as well as barotropic processes), perhaps even during its formation. Figures 1 and 2 tend to confirm this, by showing first, a concentration of rain—implying ascent and release of latent heat—in the north-west semicircle of the storm, which available temperatures and winds indicate was the warmer half between 850 and 700 mbar, and second, the presence of heavy rain at least 2 days before the vortex was detectable in low level winds.

For the Arabian Sea as a whole, the percentage coverage by rain of light, moderate or heavy rain classes is shown in Fig. 3.

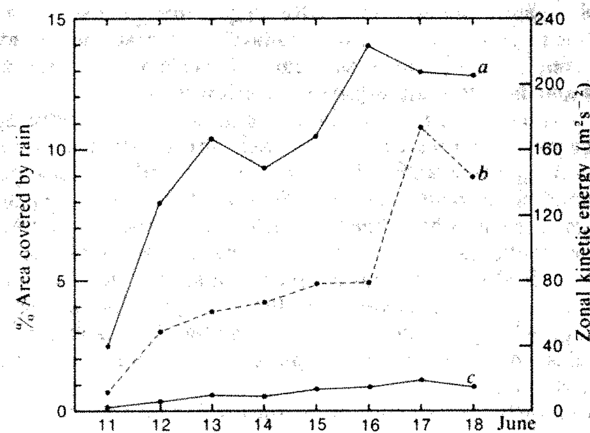

Fig. 3 Rain coverage over the Arabian Sea from 11 to 18 June. Each point is an average over the hours from 00.00 to 23.00 GMT. *a*, All rain classes. *c*, Heavy rain class only. *b*, Values of area-averaged zonal kinetic energy, from ref. 8.

The change over these eight days is remarkable: from 1% on 11 June to 14% on 18 June. The contribution of the heavy rain class to this growth is also shown in Fig. 3, from 0 to 1%. For heavy rain especially, the coverage curves closely parallel changes observed by Krishnamurti *et al.*[8] in zonal kinetic energy averaged over the Arabian Sea, suggesting that the convective response of the atmosphere to accelerations of the low level flow was relatively fast.

We conclude that the technique described here can provide information on the changing pattern of rainfall over the Arabian Sea through the onset phase of the summer monsoon. The precise limits of its accuracy remain to be established.

During the 1979 onset a near-equatorial belt of rainfall spread northwards across the centre of the Arabian Sea. Heavy rains developed, in association with an onset vortex, on the north flank of the Somali jet and moved north as the jet moved north.

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Marine snow: major site of primary production in coastal waters

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Fragile aggregate particles, or 'marine snow', were collected by SCUBA from coastal surface waters using trace metal-free techniques, and their primary production (rates of photosynthesis by associated phytoplankton) was determined relative to corresponding water column production. Primary production associated with these particles was found to range from 11 to 58% of total production, indicating that large particles can have an important role as major sites of primary production in the marine environment. Our results suggest that the use of water bottles to collect samples for productivity measurements may not sample large particles adequately in certain conditions, and thus may lead to underestimates of primary production.

Macroscopic aggregates in the millimetre–centimetre size range are a common feature of the pelagic ecosystem. Compositionally, 'marine snow' is very complex and includes both organic and inorganic materials. Several origins for these amorphous aggregates have been suggested: adsorption of dissolved and colloidal carbon onto bubbles or other surfaces[1]; mucus flocs derived from various zooplankton such as the houses of larvaceans[2] or mucus webs produced by pseudothecomatous pteropods[3]; agglutination of fecal material or zooplankton remains[4]; or mucus colonies or mats formed from various types of phytoplankton.

Because of their fragile nature, the use of conventional collection techniques (such as nets or water bottles) has usually resulted in disruption of these systems such that direct study was precluded[5]. Recently, techniques involving direct hand collection of marine snow in conjunction with SCUBA[6,7] have provided relatively undisturbed samples of these particles which are amenable to laboratory analysis. Macroscopic aggregates serve as enrichment sites for chlorophyll[6], various organic compounds such as proteins, carbohydrates and lipids[7], microscale nutrient patches[8] as microhabitats for various micro- and macro-organisms[9], and as sites of metal concentration[10]. In addition, relative aggregate primary production has recently been determined from samples collected in the southern California Bight[11]. Aggregate production values relative to total ranged from 0.1 to 9%. These are lower than the values we report here. This could reflect either regional differences in productivity, much lower (0.1–1.1 aggregates l^{-1}) particle concentrations, the use of distilled water to rinse productivity filters, or the fact that metal-free techniques were not used.

Recent work with sediment traps indicates that large particles represent an important fraction in terms of living biomass throughout the water column[12]. These large particles are not typically collected using water bottles[13,14], and can only be collected using sediment traps (except in surface waters where some sampling by SCUBA is possible). Because of the large particle–living biomass relationship, and because water bottle collections do not adequately sample such large particles, we felt that a significant portion of phytoplankton primary production (given the biological and chemical associations) may be overlooked. We therefore collected large particles from the euphotic zone to determine their associated rates of primary production relative to 'ambient' (that is, containing no large particles) water from the same depth. Further, we compared these results with samples collected by standard procedures which involved water collection bottles.

Samples of marine snow were collected from two stations in the area of Monterey Bay, California in June and July 1981 from a depth of 10 m using SCUBA techniques similar to those devised for open-ocean blue-water diving[5,6]. Aggregate abundance (Table 1) was determined using a 2.3-cm diameter loop mounted on a TSK flowmeter[6]. As is often the case with marine snow particles, particle size at a given station was remarkably homogenous. Aggregates from the June collections were all relatively large and well formed, while aggregates collected in July were smaller, more loosely consolidated, and appeared to be in an earlier state of development. Note that stringent anti-contamination methods were used during all phases of sample collection and manipulation[10,15,16], as significant reduction in primary production measurements can occur from metal

Table 1 Contribution of diver-collected marine snow aggregates to total primary production

Collection date	Mean no. (aggregates l^{-1})	Fractionated production ($\mu g\,C\,l^{-1}\,h^{-1}$)			Niskin production* ($\mu g\,C\,l^{-1}\,h^{-1}$)	Aggregate production (% total)	Niskin production (% total)
		Aggregate	Ambient	Total†			
June 1981	11.5±3.1‡	11.8	11.8	22.1	14.1±4.7	53.4	63.8
	$n = 10$	14.0	13.7	24.3		57.6	58.0
		11.4	8.4	21.7		52.5	65.0
		—§	7.4	—		—	—
		$\bar{X}$ 12.4	10.3	22.7		54.5	62.3
		± 1.4	2.9	1.4		2.7	3.7
July 1981	6.6±2.8	2.3	12.0	16.1	17.3±1.6	14.3	107.
	$n = 10$	1.8	12.2	15.6		11.2	111.
		8.5	16.1	22.3		38.3	77.6
		5.0	14.8	18.8		26.7	92.0
		$\bar{X}$ 4.4	13.8	18.2		22.6	97.0
		± 3.1	2.0	3.1		12.4	15.0

Total production is also compared with primary production values derived from Niskin bottle collections.

* Samples collected and processed using trace metal clean techniques[17].

† Total indicates primary production derived from the sum of diver-collected aggregate and mean ambient (containing no aggregates) water fractions.

‡ All errors are ± 1 s.d.

§ Sample lost.

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contaminants[16]. Aggregates were collected in 3–7 m lengths of acid-cleaned 9.5 mm diameter Bev-A-Line tubing. Before collections, the ends of the collection tubes were connected to form a continuous loop and placed into acid-cleaned plastic bags to avoid contamination. Polyethylene gloves were worn underwater while sampling and aggregate collection was directed into any perceivable current to avoid diver-related contamination. Sampling tubes were filled with either 15 (July collection) or 25 (June collection) aggregates per tube. In addition, at each station four tubes were filled with ambient seawater only, to assess the productivity of this fraction and also to correct for the inclusion of ambient seawater in the aggregate collection tubes. After collection, each sampling tube was reconnected, placed in its respective plastic bag, and returned to the ship for processing. Aboard ship, the contents of each tube (four tubes containing aggregates and ambient seawater and four tubes containing only ambient seawater) were emptied into 250 ml acid-cleaned polycarbonate flasks, injected with 10–20 μCi $H^{14}CO_3^-$ and incubated for 3 h in a seawater cooled deck-top incubator designed to simulate light levels at 10 m. Following the incubation period, samples were filtered and the filters counted by liquid scintillation to determine the amount of photosynthetically fixed carbon. The primary production procedure used followed clean techniques described in ref. 16.

At the same time as particle collection, water samples from each station were obtained from a depth of 10 m using standard 5-l Niskin bottles. These had been modified and acid cleaned to preclude metal contamination and mounted on non-metallic synthetic hydroline[17]. Incubation, processing and counting of these samples followed the same procedure described above for the aggregate samples.

The results are presented in Table 1. It is obvious from the fractional production data that aggregate contribution to primary production can represent a significant portion of the total production. Thus, during the June sampling period when the aggregates appeared well formed and rather abundant (11.5 aggregates l^{-1}), >50% of the total production was associated with these particles. In contrast, during the July sampling period, the aggregates were less concentrated (6.6 aggregates l^{-1}), more loosely consolidated and probably in an earlier stage of development. Here, the aggregates contributed relatively less to total production with values ranging from 11 to 38%.

Another interesting aspect of this study, and a potentially serious problem, involves comparing total primary production derived from hand-collected samples with Niskin bottle collections (Table 1). It is apparent that, depending on the kinds of particles present in the water column, significant errors can result due to the failure of the bottles to sample the particle field adequately. For example, during the June sampling period, primary production calculations derived from Niskin bottle samples could have underestimated production by 40% (Niskin production = 62% of the total fractionated production). However, in July, when the particles were less consolidated, there was no significant difference between SCUBA and bottle-collected samples. Thus, experimental design flaws of this sort can not only lead to underestimates of this important parameter, but may also contribute to overall sampling variability.

For an accurate understanding of primary productivity processes in marine ecosystems, it is imperative that the role of large particles as well as efficient procedures for sampling these particles be established.

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Tulu Bor Tuff at Koobi Fora correlated with the Sidi Hakoma Tuff at Hadar

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The Hadar Formation of Ethiopia is renowned for its superb mammalian fossils, but there is still some doubt about the age of the Hadar faunas and their relations to faunas from other sites despite intense efforts to date the Hadar material[1,2]. Here I report a stratigraphical tie between the Hadar Formation and formations in the Lake Turkana region, which helps to relate fossil faunas from the two areas.

The fossil site of Hadar is located along the lower reaches of the Awash River; the Shungura and Usno Formations are located in the lower Omo valley north of Lake Turkana, and the Koobi Fora Formation is located on the north-east side of that lake. The fossiliferous formations of the Lake Turkana region have many tuffs in common, and probably derive from a single depositional system dominated by the Omo River[3,4]. The Awash River and the Omo River have a common drainage divide for a distance of ~100 km through the Guraghe Mountains northwards to Badda Rogghie. According to Mohr[5], the Guraghe Mountains "are composed solely of silicic lavas and tuffs except for the summit line which is formed of superimposed massive trachyte". The age of these lavas and tuffs is unknown, but if volcanoes in the general region of Mt Guraghe erupted during the Plio-Pleistocene, eruptive products might have found their way both to the lower Awash Valley, and into the Lake Turkana Basin.

A prominent tuff in the Koobi Fora Formation is the Tulu Bor Tuff, which is correlative with Tuff B of the Shungura Formation, and Tuff U-10 of the Usno Formation[3]. These tuffs are of normal polarity, and are thought to lie in the lowest normal part of the Gauss Normal Epoch, thus having an age between 3.17 and 3.42 Myr (ref. 6). Two minor chemical variants of the Tulu Bor Tuff have been recognized, which correspond to Tuff B-α and Tuff B-β of the Shungura Formation[3]. Walter[1] provides analyses of shards from the Sidi Hakoma

Table 1 Comparative analyses of the Sidi Hakoma Tuff and Tulu Bor Tuff microprobe analyses of major and minor elements (wt %)

	Sidi Hakoma range	Tuff* average	β-Tulu Bor Tuff†
SiO_2	71.70–73.23	72.54	71.69
TiO_2	0.12–0.24	0.16	0.14
Al_2O_3	12.16–12.60	12.37	11.92
Fe_2O_3‡	1.24–1.48	1.33	1.39
MgO	0.00–0.06	0.04	0.05
CaO	0.24–0.33	0.29	0.28

* From ref. 1. † From ref. 12. ‡ Total iron expressed as Fe_2O_3.

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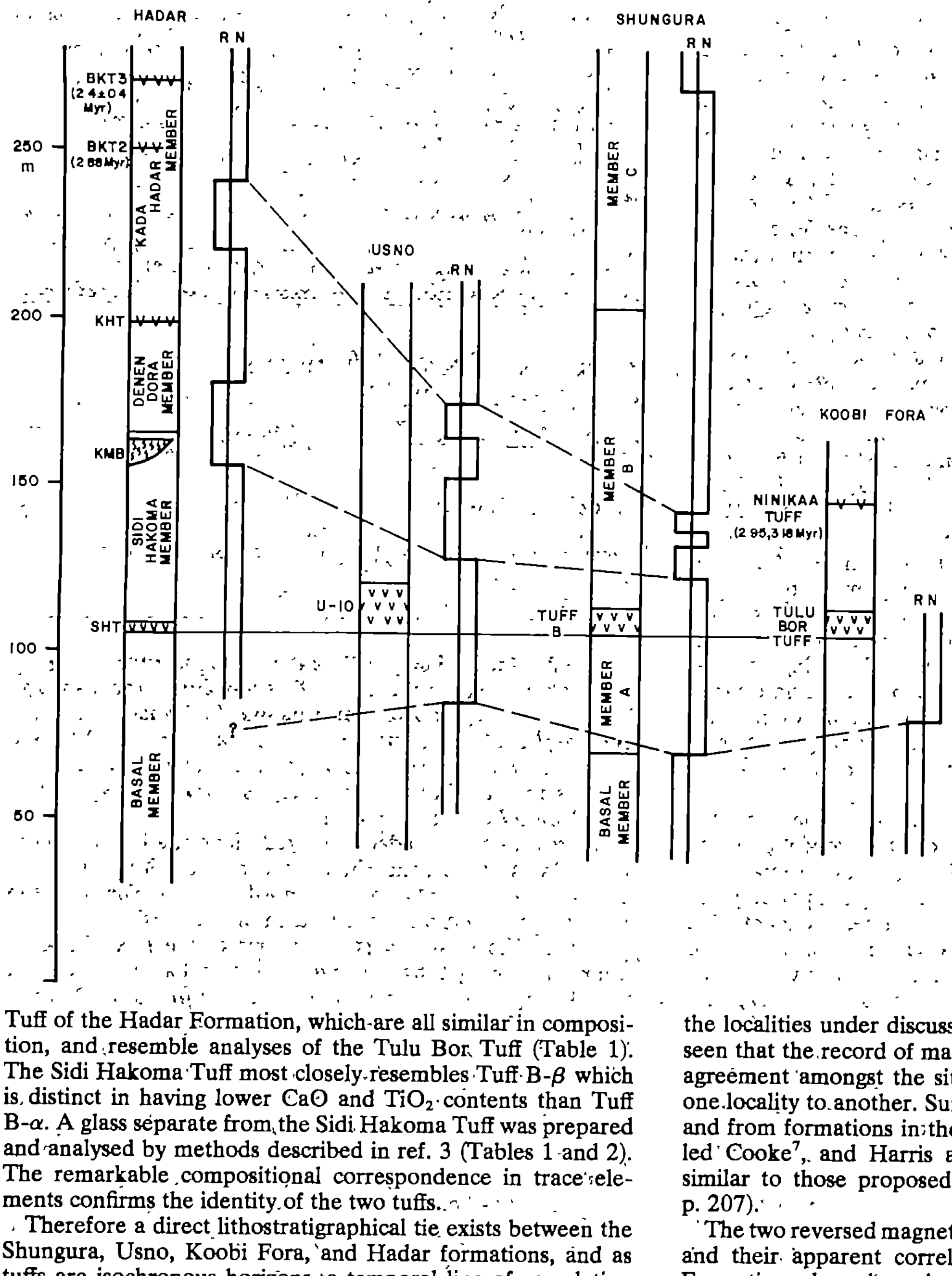

Fig. 1 Schematic partial stratigraphical columns for the Hadar, Usno, Koobi Fora and Shungura Formations. The magnetic polarity log for the Koobi Fora Formation is from ref. 13.

Tuff of the Hadar Formation, which are all similar in composition, and resemble analyses of the Tulu Bor Tuff (Table 1). The Sidi Hakoma Tuff most closely resembles Tuff B-β which is distinct in having lower CaO and TiO_2 contents than Tuff B-α. A glass separate from the Sidi Hakoma Tuff was prepared and analysed by methods described in ref. 3 (Tables 1 and 2). The remarkable compositional correspondence in trace elements confirms the identity of the two tuffs.

Therefore a direct lithostratigraphical tie exists between the Shungura, Usno, Koobi Fora, and Hadar formations, and as tuffs are isochronous horizons, a temporal line of correlation also extends to each of these localities. Schematic sections of the localities under discussion are shown in Fig. 1, where it is seen that the record of magnetic polarity transitions is in good agreement amongst the sites, although thicknesses vary from one locality to another. Suid fossils from the Hadar Formation and from formations in the Lake Turkana Basin have already led Cooke[7], and Harris and White[8] to suggest correlations similar to those proposed here (not as misquoted in ref. 9, p. 207).

Table 2 X-ray fluorescence analyses of minor and trace elements (values in p.p.m. except where noted)

	SHT-106†	80-177‡	Range β-Tulu Bor Tuff (16 samples)
CaO (%)	0.32	0.31	0.28–0.36
Fe_2O_3* (%)	1.56	1.58	1.45–1.77
Ba	144	134	112–256
Mn	381	392	351–435
Nb	84	85	78–86
Rb	110	111	108–126
Sr	8	5	5–16
Ti	884	987	877–1147
Y	61	62	57–65
Zn	86	77	72–100
Zr	369	375	351–453

* Total iron expressed as Fe_2O_3.
† Sidi Hakoma Tuff.
‡ β-Tulu Bor Tuff, Area 117, Koobi Fora.

The two reversed magnetozones above the Sidi Hakoma Tuff, and their apparent correlatives in the Shungura and Usno Formations, have been independently identified as the Mammoth and Kaena Events[6,10]. Such identifications are consistent with all K/Ar and fission-track ages from the Hadar Formation with the exception of the 3.6-Myr age on the Type B Kadada Moumou Basalt (KMB). The mean of all ages on the Type A KMB is 3.01 Myr, which when corrected upward by 3.5% (see statement in ref. 11) would be 3.12 Myr, in concordance with its reversed polarity. These identifications are also in accord with K/Ar dates on tuffs from the Lake Turkana Basin except for the dates on Tuff B-10 of the Shungura Formation, which are apparently too old (see ref. 6).

As most of the fossil material at Hadar derives from levels between the Sidi Hakoma Tuff and BKT-2 (see Fig. 1), which is only a few metres above the top of the Kaena Event, these faunas must correlate with faunas from the lower part of Member B of the Shungura Formation, and with those of the Usno Formation. It is likely that these faunas all lie between 3.3 and 2.8 Myr in age. This chronology is consistent with the palaeomagnetic record at Hadar and in the Lake Turkana Basin, with the suid evidence, and with all radiometric age determinations except those noted above. The mean sediment accumulation rate for the interval between the Sidi Hakoma Tuff and BKT-2 is ~35 cm kyr^{-1}, which is also reasonable. The ages on the Type B KMB are evidently too old, but this discrepancy has not yet been explained.

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Faunal age of the Usno, Shungura B and Hadar Formations, Ethiopia

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Recent work by Brown[1], correlating the Sidi Hakoma (or Sidiha Koma[2]) Tuff at Hadar with Tuffs B and U-10 of the Shungura and Usno Formations, respectively, reverses a recent finding[3] indicating an age of the Hadar Formation fauna of 2.9 to greater than 3.6 Myr BP. The difference in these determinations is 0.4 Myr, a significant period of evolutionary time. We compare the faunas from Shungura Unit B10, Usno and Hadar and discuss the implications for interpretation of the early hominid fossil record.

The fauna from Member B of the Shungura Formation derives primarily from the uppermost units, particularly Unit B10, superjacent to the B10 Tuff dated at 3.03 ± 0.07 Myr BP[4]. The faunal sample from White Sands and Brown Sands localities of the Usno Formation, which has been correlated with lower Member B (Unit B2), is situated stratigraphically near Tuff U10 dated at 3.05 ± 0.30 Myr BP[4] and above the basalt of U1 dated at 3.39 ± 0.23 Myr BP[4]. The Shungura B10 fauna occurs above the top of the Kaena Event (2.92 Myr BP), and the Usno fauna straddles the bottom of the Mammoth Event (3.15 Myr BP) but is younger than the Gilbert–Gauss boundary at 3.4 Myr BP[5]. Using the K/Ar and palaeomagnetic determinations to estimate best-fit ages of 2.92 and 3.15 Myr BP for B10 and Usno/Lower B faunas, respectively, it is clear that they are separated by ∼0.2 Myr. Considerations of the age of the Shungura Member B fauna therefore must include a realization that there is significant time difference between upper (B10) and lower (B2) portions of the member, the latter equivalent in time to Usno.

Table 1 gives large mammal species lists for Shungura B10, the Usno Formation and the Hadar Formation, compiled from published sources[6–13], the Omo faunal catalogue and our own interpretations, except where indicated. Table 2 summarizes results from six faunal similarity coefficient calculations. By any method the Usno and Hadar faunas are more similar than are Shungura B10 and Hadar faunas, thus indicating a probable age for Hadar nearer to 3.15 Myr BP than to 2.92 Myr BP.

Comparison of similarity coefficients based on presence or absence of taxa at Hadar with faunas in the range 3.2–4.0 Myr BP, such as at Laetoli, Kanapoi, Mursi and Ekora, is hindered by the small and not fully studied or published faunal inventories from the latter sites. However, by a consideration of evolutionary stages of single taxa, particularly in this case, those at Hadar not shared with Usno, biostratigraphical evidence can be obtained. The species not shared are: *Parapapio* cf. *jonesi*, *Theropithecus darti*, *Canis* aff. *mesomelas*, *Pachycrocuta* aff. *perrieri*, *Loxodonta adaurora*, *Elephas recki brumpti* (ex-'Stage 1'), *Ceratotherium* cf. *praecox*, *Kolpochoerus afarensis*, *Giraffa stillei*, *Praedamalis deturi*, and *Madoqua* ?*avifluminis*. Unshared generic identifications at Hadar of potential biostratigraphical importance are *Mammuthus* sp., *Ugandax* sp. nov., ?*Pelorovis* sp. and Ovibovini sp. aff. '*Bos*' *makapaani*.

The first four species listed and *Elephas recki* occur in the South African middle to late Pliocene site of Makapansgat[14], as do three other shared Hadar-Usno species. Unshared '*Bos*' *makapaani* from Buffalo Cave, Makapansgat may be of similar age. *E. recki* and ?*Pelorovis* sp. are Hadar taxa not shared with Usno but present in the later Shungura B10 fauna. *Hipparion afarense* is more primitive than *H. ethiopicum*, which first occurs in Shungura Formation Member C, but is more advanced (and apparently later in time) than *H. sitifense* found in both Shungura B10 and Usno faunas[16]. *Loxodonta adaurora* and *Ceratotherium praecox* occur in the Omo Mursi Formation, at ∼4 Myr BP[7]. Cooke[9] has discussed *Kolpochoerus afarensis* as ancestral to, but especially as seen in the larger specimens, very similar to *K. limnetes*, found in Usno and B10 assemblages. *Giraffa stillei*, although known from Laetoli, is considered by Churcher[17] to range from the "?early Pliocene to early middle Pleistocene" and it may prove synonymous with *G. gracilis*, present at Usno and Shungura B10. Gentry[10] considers *Praedamalis deturi* suggestive of "an older age than Shungura upper Member B" and Hadar *Madoqua* ?*avifluminis* similar to specimens from the Ndolanya Beds at Laetoli. The Ndolanya Beds are dated to between 2.41 ± 0.12 and 3.49 ± 0.12 Myr BP[18], in the same age range as the Usno Formation. The genus *Mammuthus*, with three recognized species, ranges in Africa from the Pliocene to the early Pleistocene[19]. *Ugandax* sp. is "no later than Shungura upper B and probably earlier"[11].

The faunal similarities with Makapansgat, with Shungura B10 and the presence of *Hipparion afarense* are suggestive of an age younger than the Usno Formation, while *Loxodonta adaurora*, *Ceratotherium praecox*, *Kolpochoerus afarensis* and perhaps *Giraffa stillei* suggest an older age. The bulk of the diagnostic fauna, however, is consistent with an Usno/Shungura lower B age for the Hadar fauna.

This finding has several implications for interpretation of the fossil hominids from the Hadar Formation. Because the fossil-bearing Hadar Formation is constrained between SHT (∼3.2 Myr BP, based on ref. 1) on the lower end and BKT-2 (2.88 ± 0.08)[3] and the top of the Kaena Event (∼2.92 Myr BP) on the upper end, the hominids are relatively closely spaced in time. There are not more than some 300,000 yr between hominids at the top and bottom of the sequence.

The temporal correlation of Pre-Kadada Moumou (or Kada Damoumou[2]) Basalt Hadar hominids with Laetoli hominids[3] (dated now between 3.49 ± 0.03 Myr BP and 3.76 ± 0.03 Myr BP[17]) is called into question. There may be between 0.3 and 0.9 Myr difference in time between the two parts of the type-series of *Australopithecus afarensis*[20].

The similarity in time between Hadar hominids and those from Makapansgat may be closer than suspected[21]. Not only does Hadar now appear later in time, but several indications seem to place Makapansgat earlier. Cooke[14] records from this site *Notochoerus capensis*, also known from the 'Kubi Algi' Formation and possibly Kanapoi[22], both apparently near 4 Myr BP, and *Potamochoeroides shawi*. The latter is termed *Metridiochoerus andrewsi* (I) by Harris and White[23] but Cooke[22] regards it as a South African endemic Pliocene suid not particularly useful for biostratigraphical correlation. Cooke[14] does not recognize at Makapansgat the typical *Metridiochoerus andrewsi* which is found in Shungura Member G (not as reported in ref. 21). An additional indicator for the 'old' age at

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Table 1 Species lists of the large mammals from Hadar, Usno and Shungura B10

Hadar Formation	Omo Shungura B-10	Omo Usno Formation
Parapapio cf. *jonesi**	—	—
Theropithecus darti⁺	*Theropithecus brumpti*⁺	*Theropithecus* sp.
—	*Cercopithecus* sp.	*Cercopithecus* sp.
Rhinocolobus cf. *turkanaensis**	*Rhinocolobus turkanaensis**	*Rhinocolobus turkanaensis**
—	*Paracolobus mutiwa*	—
—	*Papio* sp.⁺	*Papio* sp.⁺
Galerella sp.	—	—
—	*Helogale* sp.	—
Herpestes sp.	—	—
Viverra (*Civettictis*) sp.	*Viverra* (*Civettictis*) sp.	—
—	*Viverra leakeyi*	*Viverra* cf. *leakeyi*
—	*Pseudocivetta ingens*	—
aff. ?*Poecilogale* sp.	—	—
—	—	*Lutra* sp
Enhydriodon sp. nov. A	—	—
—	*Enhydriodon* sp. nov. B	*Enhydriodon* sp. nov. B
Canis aff. *mesomelas*	—	—
Pachycrocuta aff. *perrieri*	—	—
cf. *Crocuta* sp.	—	—
Percrocuta sp.	—	*Percrocuta* sp.
—	*Hyaena* aff. *makapani*	*Hyaena* aff. *makapani*
Megantereon sp.	?*Megantereon* sp.	*Megantereon* sp
Homotherium sp.	*Homotherium* sp.	*Homotherium* sp.
Dinofelis sp.	*Dinofelis* sp.	*Dinofelis* sp.
Panthera sp.	*Panthera* sp.	*Panthera* sp.
Felis sp.	*Felis* (*Lynx*) *caracal*	—
Deinotherium bozasi	*Deinotherium bozasi*	*Deinotherium bozasi*
Loxodonta adaurora	—	—
Elephas ekorensis	—	*Elephas ekorensis*
Elephas recki brumpti	*Elephas recki brumpti*	—
Mammuthus sp.	—	—
Hipparion afarense	—	—
—	*Hipparion sitifense*	*Hipparion sitifense*
Ceratotherium cf. *praecox*	—	—
Ceratotherium simum	*Ceratotherium simum*	*Ceratotherium simum*
Diceros bicornis	*Diceros bicornis*	*Diceros bicornis*
Kolpochoerus afarensis	—	—
—	*Kolpochoerus limnetes*	*Kolpochoerus limnetes*
—	*Metridiochoerus jacksoni*	—
Notochoerus euilus	*Notochoerus euilus*	*Notochoerus euilus*
Nyanzachoerus kanamensis	*Nyanzachoerus kanamensis*	*Nyanzachoerus kanamensis*
Hexaprotodon aff. *protamphibius turkanaensis* (="Afar sp. A")	—	*Hexaprotodon* aff. *protamphibius turkanaensis* (=Afar sp. A)
—	*Hexaprotodon shungurensis*	—
Hexaprotodon sp. (="Afar sp. B")	—	—
Sivatherium maurusium	*Sivatherium maurusium*	*Sivatherium maurusium*
—	*Giraffa gracilis*	*Giraffa gracilis*
Giraffa stillei (?aff. *gracilis*)	—	—
—	*Giraffa* "*pygmaeus*"	—
Giraffa jumae	*Giraffa jumae*	?*Giraffa jumae*
Tragelaphus nakuae	*Tragelaphus nakuae*	*Tragelaphus nakuae*
Tragelaphus sp. nov	—	—
—	*Tragelaphus gaudryi*	—
—	*Taurotragus* sp.	—
Ugandax sp. nov	—	—
—	*Syncerus* sp.	—
?*Pelorovis* sp.	*Pelorovis* sp.	—
Praedamalis deturi	—	—
Kobus sp. A	—	—
Kobus sp. B	—	—
Kobus sp C	—	—
—	*Kobus kob*	—
—	*Kobus ancystrocera*	—
—	*Redunca* sp.	—
?*Damalops* sp.	—	—
—	*Megalotragus* ?*kattwinkeli*	—
—	?*Oreonagor* sp.	—
Aepyceros sp.	*Aepyceros* sp. nov.	*Aepyceros* sp. nov
—	*Antidorcas recki*	—
Gazella sp	—	*Gazella* sp.
Madoqua ?*aviflumuminis*	—	—
?*Raphicerus* sp.	—	—
Ovibovini sp. (aff. "*Bos*" *makapaani*)	—	—

Compiled from refs 6–13. *, Identification by E. Delson, ⁺, identification by G. G. Eck. Hominid taxa and identifications above the subfamilial or tribal level are not included. For the purposes of calculating the similarity coefficients in Table 2, qualified species identifications (cf., aff. and ?) have been counted as occurrences. Identifications to genus only are not counted in calculations.

Table 2 Faunal similarity coefficients among Hadar, Usno and Shungura B10 faunas

Faunal comparison	Hadar–Usno	Hadar–Shungura B10	Usno–Shungura B10
Simpson (C/N_1)	0.61	0.35	0.88
Jaccard $\left(\frac{C}{N_a+N_b-C}\right)$	0.28	0.20	0.53
Kulczynski $\left(\frac{C(N_a+N_b)}{2N_aN_b}\right)$	0.48	0.33	0.73
Burt-Pilot $\left(\frac{2C}{N_a+N_b}\right)$	0.44	0.33	0.69
Otsuka $\left(\frac{C}{\sqrt{N_aN_b}}\right)$	0.46	0.33	0.71
Braun-Blanquet $\left(\frac{C}{N_2}\right)$	0.35	0.32	0.57

Formulae are from ref. 26. N_a and N_b are observed number of species in the two assemblages, C is the number of species in common, N_1 is the smaller and N_2 the larger of N_a and N_b.

Makapansgat is a recent report by Lehmann and Thomas[24] that the Makapansgat bovid, *Redunca* aff. *darti* also occurs in an upper level of the basal Pliocene Sahabi Formation, Libya. *Nyanzachoerus kanamensis*, a species found at Hadar and Usno, occurs in this same level at Sahabi[25]. Thus it is far from clear that Makapansgat *Australopithecus africanus* post-dates Hadar *A. afarensis*. Hadar and Makapansgat may be broadly equivalent in faunal age to the Usno Formation, although Makapansgat probably ranges later in time.

Because of the poor knowledge of African faunas in the 3.2–4.0 Myr BP time range, it is difficult to reject categorically a pre-Usno age for Hadar. Nevertheless, our results are consistent with the age suggested by Brown[1] for the SH Tuff at Hadar.

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Evidence that seals may use echolocation

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Harbour seals (*Phoca vitulina*), like many phocid species, forage to some extent at night[1]. Although nocturnal mammals typically rely on non-visual sensory channels, seals have adapted to low light levels by sharpening their visual sense[2]. Although enhanced visual sensitivity would be functional in clear water, many species inhabit turbid coastal or estuarine regions where vision is sometimes of limited use even in daylight. Here we present experimental evidence in support of the contention that when visual cues are not available, seals use echolocation. Our results help to explain why previous attempts to demonstrate sonar abilities in pinnipeds have been unsuccessful.

Circumstantial evidence suggests that pinnipeds use some form of echolocation. Some species produce click vocalizations similar to the signals of animals that are known to have a sonar system[3]. Harbour seals click more frequently in the dark[4] and there are numerous reports of blind seals functioning sufficiently well to raise a pup[5]. However, all experimental evidence has been negative. Evans and Haugan[6] reported that although a sea lion (*Zalophus californianus*) was able to retrieve rings at night, no vocalizations were recorded, and the animal refused to perform when blindfolded. Schusterman[7] tried to train *Zalophus* to discriminate between visually identical plexiglass disks, one filled with air, the other solid so that each disk would return a different echo to the animal. After over 2,000 trials the sea lion was unable to distinguish reliably between the targets, even though click trains were recorded on most trials. Scronce and Ridgway[8] trained a blindfolded grey seal (*Halichoerus grypus*) to retrieve a ring, and later presented the seal with the task of discriminating between two differently sized styrofoam surfaces. These authors concluded that the seal was not using sonar because few clicks were recorded during retrieval, and because the animal, having produced no vocalizations, was unable to learn the styrofoam discrimination.

Our recordings in the field[4] and from captive animals indicate that harbour seals produce at least two types of signals. The first is a brief, rapid-onset pulse which is emitted either as a single click or as a doublet (see Fig. 1*a*). Because of its fast onset, the click spans the entire audio spectrum, however most of the energy lies near 7.5 kHz. The second signal consists of a train of these clicks emitted in rapid succession such that the interval between each one determines the overall pitch of the burst (see Fig. 1*b*).

At Miquelon (45° 45′ N, 56° 14′ W) where there is a breeding colony of *P. vitulina*, we found that both vocalizations were

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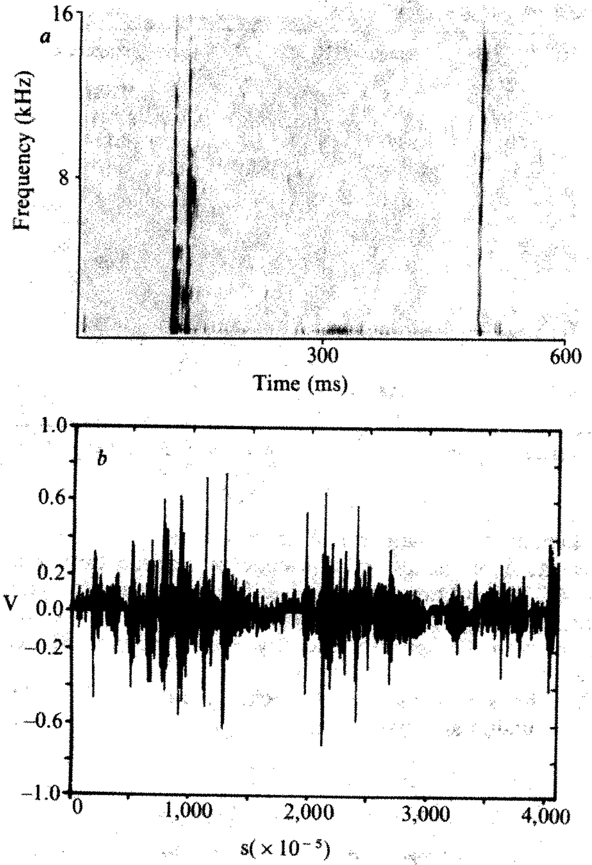

Fig. 1 *a*, A sonagram of a single and a doublet click made by the seal during discrimination training. *b*, Waveform of a train of clicks recorded from a breeding colony of harbour seals at Miquelon.

more frequent at night. However, before the present experiment, our captive harbour seals would only click when given live fish in total darkness. In early attempts to use Schusterman's procedure[7] with a harbour seal, we found that the animal would not click in the presence of the disks, perhaps because it had no understanding of the fact that echoes might be of assistance. We then modified the method to enhance the likelihood that the seal would use sonar to solve a similar discrimination problem.

The subject of the experiment was a 7-yr-old male harbour seal who has lived in captivity since it was collected as a weaned pup. The apparatus consisted of two rings made of hollow black plastic tubing, 4 cm in cross-section. The inner diameter of each ring was 9 cm, and the outer diameter 17 cm. One ring was internally ballasted with 600 g of lead; the other contained 101 g of lead and was filled with water. Both rings were negatively buoyant and during the second phase of the experiment were suspended 0.5 m apart in the water by 1 m-long strings such that the ballast lay at the bottom of each ring. The string of the air-filled ring had a small loop at its free end so that we could distinguish between each ring during testing.

The study was done in an outdoor 7.5-m diameter tank above ground, constructed from 6.5-cm white pine. It was filled with seawater to 1.2 m depth. For certain parts of the experiment a black vinyl tarpaulin, suspended over a superstructure ~1 m above the tank, was draped to the ground so that we could work under it.

A Gould model CH-17U hydrophone was used to monitor vocalizations. During the second phase of the experiment it was positioned at the same depth as the rings, ~1.5 m to the side of the nearest ring. Signals were recorded with a Sony model TC-D5 cassette tape recorder, and a Racal Store 4 instrumentation recorder was used to determine any ultrasonic components.

The seal was trained to retrieve the air-filled ring to earn a piece of herring. The ring was slipped into the water on one side of the tank, while the seal waited, with its head out of the water, at a station on the opposite side until one of us said "fetch". The seal then located the ring, put its snout through the centre and in this way carried it back to the experimenter and tossed it over the side. Once the animal was able to do this, it was required to do so at night when the tarpaulin was in place to eliminate ambient light. We recorded the latency between the "fetch" call and the moment when the seal returned with the ring.

After seven sessions of 10 trials each, the seal was then presented with two rings, and required to retrieve the air-filled one. In this case, one experimenter held the free end of the strings to suspend the rings ~0.6 m below the water surface, ~0.8 m away from the tank wall. When the seal chose the air-filled ring, it was released and the animal brought it across the tank to the other experimenter who rewarded it with a piece of fish. If the water-filled ring was chosen it was pulled off the seal's snout, and both rings removed from the water, at which time the seal was given a 30-s rest period as 'punishment'. At least 20 trials were run in each session, and the position of the correct ring (left as opposed to right) was varied at random. After seven sessions run in the dark, the training was continued in daylight as the rings were visually identical. When the seal learned the discrimination, the air-filled ring was ballasted at 101 g and filled with water, and five additional sessions were run. Vocalizations were monitored during all sessions with the Sony recorder, and on three occasions with the Store 4.

During the first phase of the study, when the seal was required to find one ring in the dark, the animal returned it to the experimenter in, on average, 34 s (s.d. = 50.47), although the time it took to locate the ring was slightly less than this. Single and doublet clicks were emitted throughout each session, and click trains were recorded for ~40% of the trials.

During the first session of discrimination training when the seal had to identify the air-filled ring, the correct ring was chosen on 63% of the trials. This level of performance improved until the seal was able to respond correctly on at least 75% of the trials ($t = 2.34$, $P < 0.05$ for trials 1–13 compared with 14–26), the best discrimination ratio being 0.85. The only time the animal responded at less than 50% was in a few sessions just after we switched to training in daylight (see Fig. 2).

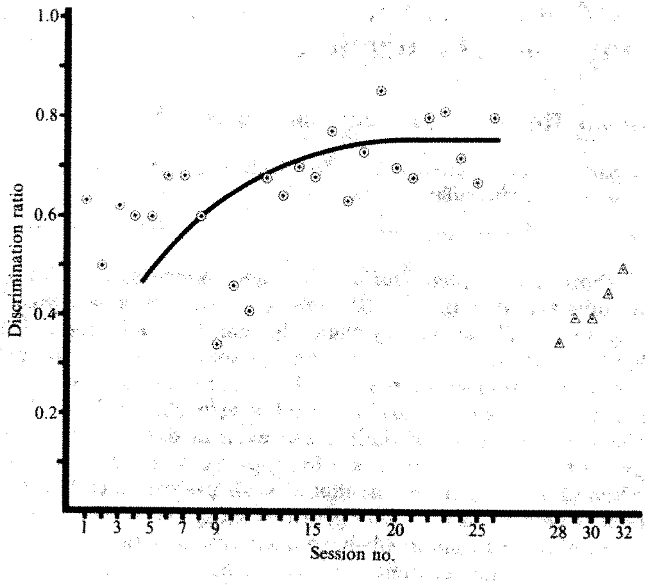

Fig. 2 Performance of the seal during discrimination training (⊕) and after both rings were filled with water (△). The discrimination ratio is the percentage of trials in which the seal chose the correct ring. Training occurred in darkness for the first seven sessions, and thereafter in daylight. The line was fitted by eye.

After 26 training sessions, both rings were filled with water and the seal's performance fell to chance levels ($\bar{x} = 0.42$, s.d. $= 0.06$, $t = 6.82$, $P < 0.05$ for control trials compared with the last five discrimination trials; see Fig. 2).

The only vocalizations that were consistently produced during discrimination training were very faint single or doublet clicks. No click trains such as those emitted during the location task were recorded. There was no evidence of an ultrasonic component to either type of signal.

The following results suggest that the seal was using echolocation: (1) it was able to locate a ring in total darkness; (2) it could discriminate between two objects that were visually identical but which differed in acoustic impedance; (3) the seal produced click vocalizations during the performance of both tasks; and (4) when the same rings were both filled with water, the animal was no longer able to distinguish between them even though it clicked while attempting to retrieve the correct one.

In our pilot study, we were unable to train a harbour seal using the same procedure as that used by Schusterman[7]. We found, as did Scronce and Ridgway[8] and Evans and Haugan[6], that the seal did not click during testing. This was perhaps not surprising as there was no reason to expect the animal to realize the task could be solved by using sonar. We therefore introduced the intermediate step of requiring the animal to first locate one ring in the dark, a situation in which it might be predisposed to using echolocation. For similar reasons we ran the first few sessions of discrimination training in the dark. Throughout the seal's early training sessions, it chose the air-filled ring correctly on about two-thirds of the trials, and only scored <0.50 after we switched to daylight testing. It is tempting to speculate that in the dark when the visual similarity of the rings was not apparent, the seal favoured the air-filled one because it was most salient, returning the loudest echo. However, the discrimination ratios in the seven sessions in the dark and for the first seven in daylight are not statistically different ($t = 0.79$, $P > 0.05$), and it is therefore impossible to determine whether the change in the seal's behaviour was a function of the rings being visible or a reflection of performance instability during early training. Similarly, the increase in the discrimination ratio from 0.35 to 0.50 over the sessions when the seal was presented with two water-filled rings is not statistically significant ($x^2 = 1.32$; $P > 0.05$).

The seal seemed to find the discrimination task difficult; perhaps its sonar system is not sophisticated enough to easily make distinctions like the one we devised. Most proficient echolocators operate primarily in the ultrasonic range as the higher the carrier frequency and/or pulse repetition rate, the better the resolution of the system. Certainly, an echolocator having a low frequency signal would be at a disadvantage in an enclosed tank in which reverberant surfaces are close, and ambient noise high. Quiet individual or doublet clicks emitted after perceptible delays might have been the seal's solution to the problems of reverberation, especially during discrimination training when the rings were close to the tank wall.

Although our experiment provides evidence of echolocation, a study of this type cannot eliminate unequivocally the possibility that some cue of which we were unaware, may have somehow assisted the seal. Norris[3] cautioned that the only way to unequivocally demonstrate echolocation is by blocking the animal's sound reception or emission during discrimination performances. To date, no marine mammal has willingly participated in such a procedure and as Norris goes on to admit, the general acceptance of dolphin sonar abilities rests on "less than perfect experiments". If some method of performing the critical test could be devised which would ensure that the animal was not disrupted in a general way, all doubts about echolocation in pinnipeds could be allayed once and for all.

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Isolation of infective tomato bushy stunt virus after passage through the human alimentary tract

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Research on plant virus vectors has shown that almost all types of organisms feeding on or parasitizing infected plants, including sucking and biting insects, mites, nematodes and chytrid fungi, can act as specific vectors. Surprisingly, however, natural vectors have not been found for some of the most infectious of the plant viruses such as tomato bushy stunt virus (TBSV) and tobacco mosaic virus (TMV). Here we report that when purified TBSV was consumed by human volunteers, it subsequently occurred in their faeces and could be detected by mechanical inoculation of faecal extracts to the leaves of an indicator plant (*Chenopodium quinoa*) in which the virus causes countable, discrete lesions. Thus, the virus remained stable on passage through the alimentary tract. The significance of man as a carrier, able to disseminate the virus widely within the environment, is discussed in relation to the epidemiology of the disease. To our knowledge, this is the first demonstration of an 'alimentary-resistant' plant virus.

TBSV was first isolated[1] from tomato (*Lycopersicon esculentum*) crops in Ireland in 1935 and was later found in tomato crops in England[2]. The virus occurs in nature as a number of different pathogenic but serologically-related strains infecting a variety of crops and other plant species[3] including tomato, pepper, globe artichoke, cherry, hop, grapevine, *Pelargonium* and *Petunia*. Recently, it has caused economically important tomato diseases in Morocco[4] and Tunisia (C. Cherif, personal communication) because diseased plants are stunted and the 'mottled' fruit is unacceptable for export. The distribution of TBSV includes western and central Europe, North Africa, Argentina, Canada and Mexico.

TBSV is a single-stranded RNA-containing virus with isometric particles ~30 nm in diameter. In some experimental hosts (for example, *Nicotiana clevelandii*) it may reach a concentration of ~200 mg per kg of infected leaf. It is readily transmissible by mechanical inoculation and infective leaf

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Table 1 Virus recovery from faeces of three volunteers 1–5 days after ingesting TBSV

Treatment	Volunteer	Dilution of sample	Infectivity* of faecal samples on day: 0†	1	2	3	4	5
TBSV in distilled water	T.F.	0	0	8	5	0	0	0
		1/10	0	5	2	0	0	0
	J.F.	0	0	>100	28	3	1	0
		1/10	0	92	16	2	1	0
	J.G.	0	0	>100	19	32	0	0
		1/10	0	>100	10	23	0	0
Distilled water	M.J. E.B. A.C.	0	0	0	0	0	0	0
		1/10	0	0	0	0	0	0

Three volunteers consumed 10 mg purified TBSV in 50 ml distilled water and three volunteers (controls) received water alone. TBSV was purified from systemically infected *N. clevelandii* leaves by *n*-butanol (8.5% v/v) clarification of leaf homogenates in 0.5 M potassium phosphate buffer (*p*H 7.5) followed by differential centrifugation and density gradient centrifugation on 10–40% (w/v) sucrose columns in 0.01 M phosphate buffer (*p*H 7.5). Virus zones containing TBSV were removed, diluted in distilled water and ultracentrifuged. Virus pellets were resuspended in distilled water and stored at −18 °C. Faeces were stored frozen. Samples (2–3 g) were homogenized in 0.05 M phosphate buffer *p*H 7.2 containing 200 μg ml^{-1} penicillin, 200 μg ml^{-1} streptomycin and 10 μg ml^{-1} amphotericin in sodium deoxycholate (Fungizone). After centrifugation at 5,000*g* for 15 min to remove solids, the supernatant was centrifuged for 30 min at 100,000*g*. Faecal pellets were resuspended for 4 h in 1.0 ml 0.005 M phosphate buffer *p*H 7.5 (dilution 0) and aliquots diluted 1/10 in the same buffer. Samples (coded) were manually inoculated to carborundum-dusted *C. quinoa* leaves. Resultant TBSV lesions were counted 5 days after inoculation and recorded before the code was known.

* Mean no. of TBSV lesions in four *C. quinoa* leaves.

† Virus was consumed a few hours after the first collection of faecal samples on day 0.

extracts retain infectivity after freezing for several years[5]. The thermal inactivation point is between 80 and 90 °C.

TBSV is soil-borne[6] and, although most other soil-borne viruses have nematode or fungus vectors, no vector of TBSV is known at present[3,7]. Studies, begun in 1980, on the occurrence of plant viruses in English rivers[8] have isolated TBSV from the rivers Thames and Cam, from tributaries of the rivers Avon (Warwickshire) and Trent and also from Lake Esthwaite (Cumbria). Present investigations on the origin of TBSV in rivers have demonstrated its occurrence in consignments of symptomless tomatoes imported from Morocco to Birmingham in 1982 and in tomato plants growing extraneously in sludge at sewage works in Warwickshire and Leicestershire[9]. In an attempt to explain its presence in river water and in the tomato plants growing in sludge, we have postulated that the virus remained infective after human consumption of TBSV-infected tomato fruit and that it was then transported to rivers after treatment of infective faeces at sewage works.

An *in vitro* test showed that the infectivity of TBSV (isoelectric point, *p*H 4.1) was retained after storage at *p*H 3.0, a value similar to that of many samples of gastric juice. An homogenate of TBSV-infected *N. clevelandii* leaves in distilled water (1 g per 2 ml) was clarified by centrifugation at 5,000*g* for 10 min and aliquots of the infective supernatant fluid adjusted with 10% HCl to *p*H 3.0, 3.5 and 4.0. An unadjusted aliquot (*p*H 6.5) was used as a control. After incubation for 1 or 3 h at 25 °C, the samples were neutralized (*p*H 7.0) with NaOH, stored at 4 °C for 5–6 h to allow virus resuspension and 10-fold dilutions of the samples assayed for infectivity by mechanical inoculation of *C. quinoa* leaves. Based on the numbers of lesions formed in the leaves after 5 days, no differences in the infectivity end points were found between the acid-treated and control samples.

The *in vivo* experiment was as follows. Six adult volunteers were randomly divided into two groups. Each of three volunteers (T.F., J.F. and J.G.) consumed 10 mg of purified TBSV in 50 ml distilled water and three volunteers (M.J., E.B. and A.C.) received water alone. Each volunteer consented not to eat tomatoes for 7 days before the start of the experiment. Faeces was collected from each volunteer 1 day before and daily for 5 days after consuming the test liquids. After storage at −18 °C, the faecal collections were sampled and assayed for TBSV infectivity (Table 1). TBSV was detected in faeces of each of the volunteers receiving virus but not in those from the control volunteers. For individuals receiving virus, the infectivity was similar and high in faecal extracts of two volunteers (J.F. and J.G.) but it was relatively low in the case of the third volunteer (T.F.). The reason for this is unknown, but may be due to errors in faecal sampling.

The result demonstrates the remarkable stability of TBSV in that it retained its infectivity after ingestion and successive exposure at 37 °C to the enzymes of the alimentary tract, bile salts and wide fluctuations in *p*H (~3.0–8.8).

Retrospectively, the chances of plant viruses surviving passage through the alimentary tract have usually been considered remote. With most plant viruses this may be true as with those sometimes consumed in infected raw vegetables including lettuce mosaic, celery mosaic, carrot mottle and, in brassicas, the cauliflower and turnip mosaic viruses. This is because, in infective plant tissue extracts, these viruses would be rapidly inactivated at 25 °C in solutions of *p*H 4.0–5.0. However, not all enteric pathogens need to be acid-stable—for example, *Vibrio cholerae* is not acid-stable—thus other agents present in a quantity of food could survive transit through the stomach. The more stable plant viruses such as the tomato and pepper strains of TMV may be sufficiently intrinsically stable to resist digestion, and experiments to test their possible 'alimentary resistance' will now be done. Similarly, the resistance of viroids[10], for example, cucumber pale fruit and potato spindle tuber viroids, needs to be determined; although readily hydrolysed by pancreatic RNase *in vitro* in weak salt solutions (0.005 M phosphate buffer, *p*H 7.5), these are more resistant to the enzyme in solutions of higher molarity.

The results presented here suggest that man is a potential carrier of TBSV if he has consumed infected produce. The possibility exists that TBSV could enter rivers after sewage treatment of infective faeces but whether the virus is stable after this treatment is unknown, although its presence in tomato plants growing extraneously in primary settlement beds has been confirmed. Further dissemination might also occur by irrigation of susceptible crops with infective river water. Whether human factors (infective faeces) contribute to the incidence of TBSV in Moroccan[4] and Tunisian (C. Cherif, personal communication) tomato crops merits further study. We shall now examine other plant viruses having no known vectors for their 'alimentary resistance', as man and other migratory animals may prove to be important carriers of these viruses also.

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Specific inhibition of the morphogenesis of *Trypanosoma cruzi* by a monoclonal antibody

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***Trypanosoma cruzi*, the causative agent of Chagas' disease, is transmitted primarily by blood-sucking reduviid bugs. The insects themselves are infected by ingesting trypomastigotes with the blood of infected mammals. On ingestion by the vector, the parasites convert to epimastigotes which multiply within the lumen of the crop and midgut. In the hindgut and rectum of the reduviid, the epimastigotes transform into non-dividing metacyclic trypomastigotes which can, when passed with the faeces, infect the vertebrate host[1]. Lectins capable of binding *T. cruzi* have been isolated from different regions of the vector's intestinal tract[2,3]. It has been proposed that the interaction of these lectins with the surface membrane of the parasite regulates the morphogenesis of *T. cruzi* in the gut of the vector[2,3]. In the present study we describe the inhibition of epimastigote to trypomastigote differentiation *in vitro* by a monoclonal antibody specific for a 72,000 molecular weight cell-surface glycoprotein of *T. cruzi* and propose that this molecule may be a receptor controlling parasite differentiation in the vector.**

The transformation of epimastigotes into trypomastigotes was studied in an *in vitro* assay which is described in detail elsewhere[4]. In this system, log phase epimastigotes (Tulahuen strain) cultured in liver infusion tryptose (LIT) medium are programmed for differentiation by inoculation into Millipore filter (0.45 μm) chambers which are then implanted subcutaneously in mice. When recovered 7 h after *in vivo* implantation these organisms are still in the epimastigote stage as judged by morphological criteria as well as their sensitivity to complement lysis and binding of specific lectins[4]. Nevertheless, on transfer to Eagle's minimal essential medium (EMEM) and incubation for 17 h at 37 °C in a 5% CO_2 atmosphere, 60–80% of the parasites transform into morphologically distinct trypomastigotes. This *in vitro* transformation is not observed when epimastigotes are cultured in the same conditions in the absence of prior *in vivo* programming[4].

Table 1 Monoclonal antibody reagents tested

Hybridoma	Ig class	Reciprocal IFA titre	Reciprocal agglutination titre
WIC 59.4	IgM	10^5	10^4
WIC 14.19	IgG1	10^3	10^2
WIC 29.19	IgG1	$>10^5$	$>10^5$
WIC 29.82	IgG3	10^2	$<10^1$
WIC 53.1	IgG2a	10^2	10^1
WIC 29.26	IgG2a	$>10^5$	10^3
	Fab	$>10^5$	$<10^1$

Indirect fluorescent antibody (IFA) titres were determined by adding log dilutions of Protein A-purified monoclonal antibodies or papain-digested Fab fragments (starting concentration of approximately 5 mg ml^{-1} to epimastigotes collected from LIT medium. The parasites were then incubated for 1 h at 37 °C, washed twice and incubated for an additional 30 min at room temperature with a 1/40 dilution of fluorescein-conjugated rabbit anit-mouse heavy and light chains (Cappel). After further washing, the parasites were examined in a fluorescence microscope. Direct agglutination of parasites was assayed by adding log dilutions of purified monoclonal antibody (or Fab fragments) to LIT derived epimastigotes (5×10^6 per ml) in EMEM plus 1% bovine serum albumin in round-bottom microtitre trays. After 1 h at 37 °C, agglutination of the parasites was scored by examination with an inverted microscope.

A series of six monoclonal antibodies reactive with the epimastigote surface and an epimastigote binding lectin, wheat germ agglutinin (WGA), were tested for their ability either to inhibit or promote epimastigote differentiation in the *in vitro* assay. The monoclonal antibodies were produced by fusing P3-NS1/1-AG-4-1 myeloma cells with spleen cells from BALB/c mice immunized with glutaraldehyde fixed epimastigotes as described previously[5]. With the exception of WIC 59.4, an IgM antibody, each of the monoclonal antibodies was purified from ascites by affinity chromatography on Protein A–Sepharose[6]. The purified antibodies (unpurified ascites in the case of WIC 59.4) were found to have indirect fluorescent antibody titres ranging between 10^2 and $>10^5$ and to have parasite agglutination titres ranging between $<10^1$ and $>10^5$ (Table 1). The epimastigote binding lectin, WGA, agglutinated the parasites at concentrations as low as 8 μg ml^{-1}.

When tested in the *in vitro* differentiation assay, only one of the reagents, an IgG2a monoclonal antibody WIC 29.26, significantly influenced the differentiation of epimastigotes into trypomastigotes. As shown in Fig. 1, the purified antibody significantly inhibited parasite transformation at dilutions as high as 10^5 which is equivalent to an IgG to parasite ratio of 5 ng per 10^6 organisms. No inhibitory activity was observed with the five other monoclonal antibodies at any of the concentrations tested. The effect of a representative negative antibody (WIC 29.19) with high anti-epimastigote binding activity is shown in Fig. 1. Similarly, no inhibitiion of transformation was observed with WGA even at concentrations as high as 250 μg ml^{-1} (data not shown).

A trivial explanation of the inhibitory effect of WIC 29.26 on parasite transformation is that the antibody is trypanocidal or trypanostatic. Arguing against this hypothesis is the observation that incubation with WIC 29.26 causes no reduction in

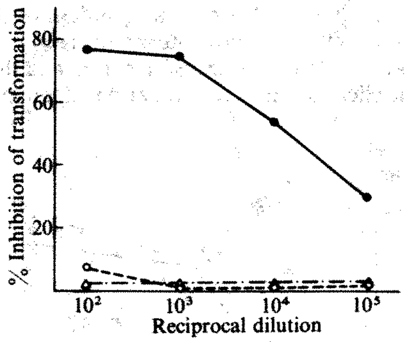

Fig. 1 Inhibition of epimastigote to trypomastigote transformation by monoclonal antibody WIC 29.26. Epimastigotes (Tulahuen strain) were collected from log phase cultures in LIT medium containing 10% fetal calf serum, washed in LIT, and inoculated into Millipore chambers which were then implanted subcutaneously into C57BL/6J mice. Seven hours later the parasites were recovered from the chambers, washed in EMEM and resuspended in EMEM to a final concentration of approximately 10^7 epimastigotes per ml. Aliquots (45 μl) of the parasite suspension were then cultured together with 5 μl of monoclonal antibody or Fab preparation in EMEM for 17 h at 37 °C in a humidified atmosphere of 5% CO_2. The contents of duplicate microwells were then smeared, Giemsa stained and the percentage of mature trypomastigotes plus partially transformed trypomastigotes determined by morphological examination[4]. In the representative experiment shown, 76% of the parasites cultured in the absence of inhibitor differentiated into trypomastigotes. The effect of the following Protein A–Sepharose-purified antibody preparations (used at an initial concentration of 5 mg ml^{-1}) is shown: WIC 29.26 IgG2a (●), WIC 29.26 Fab (△), WIC 29.19 IgG1 (○).

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epimastigote motility or in the number of total parasites recovered at the end of the 17-h assay period and that upon transfer to LIT medium after incubation in WIC 29.26 antibody parasites display subsequent growth kinetics indistinguishable from those exhibited by programmed epimastigotes or programmed epimastigotes pre-incubated with a control monoclonal antibody (WIC 29.19) (Fig. 2). Similarly, during the 17-h incubation with WIC 29.26 antibody, no inhibition of total protein synthesis (as measured by ^{75}Se-selenomethionine incorporation) is observed nor do major differences appear in the pattern of labelled proteins (as analysed by SDS–polyacrylamide gel electrophoresis) when parasites incubated in the presence or absence of WIC 29.26 antibody are compared (data not shown).

In order to investigate further the mechanism by which WIC 29.26 inhibits trypomastigote differentiation, monovalent Fab fragments were prepared from the purified antibody by papain digestion. Although the Fab preparation displayed the same indirect fluorescent antibody titre for epimastigotes as the bivalent WIC 29.26 (Table 1) it failed to inhibit transformation in the *in vitro* assay (Fig. 1).

The above findings demonstrate that monoclonal antibody WIC 29.26 potently and specifically inhibits the transformation of epimastigotes into trypomastigotes. The epimastigote surface antigen recognized by WIC 29.26 antibody has already been identified[5]. The antigenic determinant resides on the carbohydrate portion of a 72,000 molecular weight glycoprotein[5] which is present on the surface of epimastigotes and metacyclic trypomastigotes but absent on bloodstream trypomastigotes and amastigotes (D.S., unpublished data). Although the exact role of the 72,000 molecular weight glycoprotein in parasite differentiation remains to be elucidated, the failure of WIC 29.26 monovalent Fab fragments to inhibit transformation (Fig. 1) suggests that cross-linking of the molecule is essential for the biological effect.

Our data predict the occurrence of a substance in the gut of the invertebrate host with a binding specificity and biological activity similar to the WIC 29.26 monoclonal antibody. Indeed, as mentioned earlier, lectins which react specifically with different morphological stages of *T. cruzi* have already been identified in different portions of the reduviid intestinal tract[2,3]. If present in the lining of the crop and midgut of the vector, a lectin reactive with the carbohydrate moiety recognized by WIC 29.26 antibody, could inhibit transformation and thereby regulate the multiplication and accumulation of epimastigotes in this anatomical site.

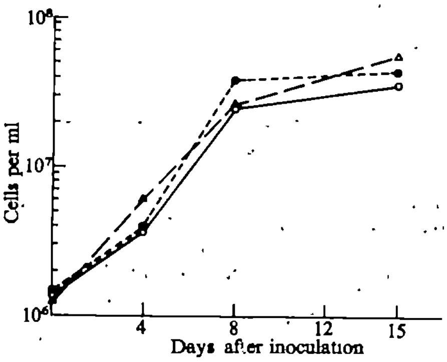

Fig. 2 Incubation with WIC 29.26 antibody has no effect on parasite viability or subsequent growth in culture. *In vivo* programmed epimastigotes incubated in EMEM for 17 h in the absence of inhibitors (○) or in the presence of a 1/1,000 dilution of either WIC 29.26 (△) or WIC 29.19 (●) monoclonal antibody were diluted 1:10 in LIT medium plus 10% fetal calf serum and the growth of the parasites assayed by haemacytometer counts. The results are the mean of assays on duplicate cultures. The absence of a significant lag in the growth of control, programmed cultures probably reflects rapid transformation of non-dividing trypomastigotes into epimastigotes during the first 4 days of culture.

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Alteration in the penicillin-binding profile of *Bacillus megaterium* during sporulation

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The development of convenient methodology to study penicillin-sensitive enzymes (PSEs) as penicillin-binding proteins (PBPs)[1,2] has revealed their roles in bacterial cell shape determination, particularly in the Gram-negative rod *Escherichia coli*[3–6]. By analysing PBPs during the life cycle of *Bacillus megaterium* KM we report here the first data suggesting specific roles for these proteins in the morphogenesis of a Gram-positive rod. During the transition from the vegetative rod to the spherical/ellipsoidal-shaped dormant spore, novel PBPs are synthesized and existing ones are proteolytically modified. The differentiation of the dormant spore PBP profile back to that of the vegetative cell during germination, follows a defined sequence clearly correlated to specific changes in shape, culminating in a highly synchronous vegetative cell division.

The sporulating bacteria offer one of the most attractive prokaryotic systems to explore the cellular role of PBPs. In appropriate growth conditions, these bacteria can be induced to undergo a limited number of central septations in a synchronous manner (vegetative growth) followed by one final synchronous septation which differs from all previous septations by being asymmetrically positioned close to one cell pole[7]. Following this unequal partitioning of the cell which is unique to sporulating cells, the membranes comprising this asymmetrical septum continue to proliferate towards the nearest cell pole with the result that a separate spherical/ellipsoidal compartment (forespore) containing at least one complete genome is created within the parent cell (mother cell)[8]. By the nature of its formation, the forespore is enclosed by two membranes of opposite polarity[9]. During the next 12 h, in a series of well defined morphological and biochemical changes programmed by transcription of spore-specific genes, this forespore is converted into a mature spore[10]. The overall sporulation process is divisible into seven distinct stages (0–VI)[11,12]. Stage II describes the asymmetric septation event and stage III denotes the first appearance of the discrete forespore. Of particular interest to the question of cell shape determination is the fact that within the cylindrical mother cell, a spherical/ellipsoidal layer of peptidoglycan (spore cortex) is synthesized between the two bounding membranes of the forespore during stages III and IV[13–16]. In addition to its altered shape, the chemistry of this cortex peptidoglycan is radically different from that of the parent cell[14,17]. Thus, the sporulating cell not only provides an opportunity to investigate the switch from symmetrical to asymmetrical division and hence to probe polarity mechanisms, but also to study the chemical and enzymatic basis of a transition from cylindrical to spherical/ellipsoidal shape. Interestingly,

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these transitions can also be investigated in reverse as dormant spores can be triggered to germinate and outgrow synchronously[8,17,18]. Approximately 180 min after germination triggering, the peptidoglycan composition has reverted to that of a typical vegetative cell, the cells have regained their cylindrical shape and the population undergoes its first synchronous central septation[16,18,19].

We analysed PBPs in membranes isolated from vegetative cells (Fig. 1*a*), mother cells (Fig. 2) and forespores during sporulation (Fig. 1*b–e*), and from dormant (Fig. 1*f*) and germinated spores (Fig. 1*g-h*). Vegetative membranes of this sporogenic *B. megaterium* KM contained five PBPs identical both in their mobilities on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and in their affinities for benzylpenicillin, to PBPs 1–5 of an asporogenic *B. megaterium* KM strain[20]. In addition, four previously undetected PBPs were identified and designated PBPs 3a, 3b (Fig. 1*a*), 3c and 3d (Fig. 1*d*). The apparent molecular weights (MWs), affinities for benzylpenicillin and specific amounts of the PBPs in vegetative and forespore membranes of *B. megaterium* KM, are shown in Table 1.

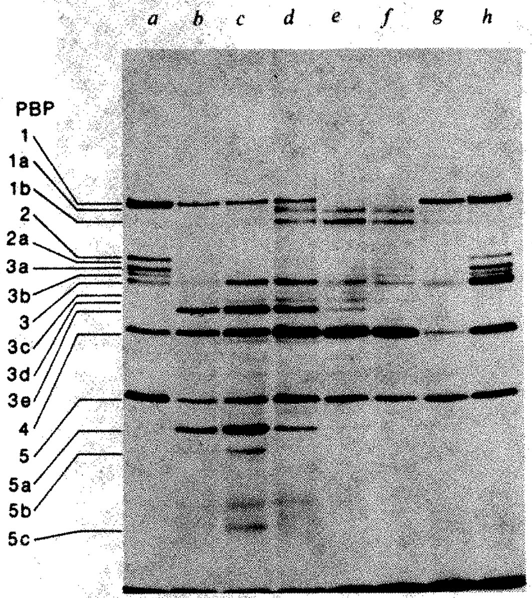

Fig. 1 Fluorogram of PBPs of membranes isolated from vegetative cells, forespores and dormant and germinated spores of *B. megaterium* KM. The conditions for growth and sporulation were as described previously[27,28]. Intact cells (exponentially growing vegetative cells, isolated forespores, dormant and germinated spores) were disrupted by homogenization with glass beads in 0.05 M Tris-HCl *p*H 7.5 buffer, containing 2 mM phenylmethanesulphonylfluoride (PMSF)[29]. Cell walls or integuments were sedimented (27,000 *g* for 3 min) and integument fractions[29] from stage V and VI forespores and dormant spores were washed four times by resuspension in 0.05 M Tris-HCl *p*H 7.5, 5 mM $MgCl_2$. The integument fractions isolated from developing and mature spores contained the outer forespore/spore membrane trapped between the proteinaceous spore coats and the cortex from stage V onwards[30–32]. The supernatant fraction was centrifuged twice (27,000*g* for 3 min) and the final supernatant was further centrifuged (105,000*g* for 60 min) to sediment membranes, which were washed twice by repeated centrifugation and resuspended in 0.05 M Tris-HCl *p*H 7.5, 5 mM $MgCl_2$ buffer. In the preparation of membranes from the sporulation stages, forespores were released after the lysozyme digestion step and the forespores and mother cell membranes isolated by differential centrifugation[28]. The temperature of all preparations was rigorously maintained below 4 °C. Freshly prepared dormant spores were germinated as described by Wilkinson *et al.*[33] in a modified sporulation medium (CCY medium) at a cell density of 0.5 mg dry weight ml^{-1}. The medium contained glutamine (50 mg l^{-1}), acid casein hydrolysate (2.5 g l^{-1}), enzymatic casein hydrolysate (2.5 g l^{-1}), yeast extract (1.0 g l^{-1}), glycerol. (1.5 g l^{-1}) plus germinants (10 mM L-alanine and 5 mM inosine). Salts, at the normal concentrations of CCY medium[27], were added 45 min after the time of transfer of spores to the medium (t_{45}). Germination, outgrowth, elongation and the first vegetative cell division were monitored by phase contrast microscopy and membranes were prepared from cells at t_{85} (elongation) and t_{200} (cell division). Membranes, at 5–10 mg protein per ml, were incubated at 37 °C for 10 min with ^3H-benzylpenicillin (25 μg ml^{-1}; 31Ci $mmol^{-1}$) in 0.05 M Tris-HCl *p*H 7.5, 5 mM $MgCl_2$ buffer. After reaction, samples were supplemented with a 1,000-fold excess of nonradioactive benzylpenicillin and an equal volume of SDS sample buffer (0.05M Tris-HCl, *p*H 7.5, 2% (w/v) SDS, 0.05 M dithiothreitol, 4 mM PMSF, 2 mM EDTA, 20% (w/v) glycerol, 0.005% (w/v) bromophenol blue), heated at 100 °C for 3 min, and subjected to 10% SDS-PAGE as described previously[29]. Equal aliquots of protein, estimated by the method of Lowry *et al.*[34], were loaded on each track. PBPs were detected by fluorography[35], using pre-flashed Fuji X-ray film. *a*, Vegetative plasma membranes; *b*, inner (ifm) and outer (ofm) forespore membranes of stage III; *c*, stage IV, ifm and ofm; *d*, stage V, ifm; *e*, stage VI, ifm; *f*, dormant spore inner membrane; *g*, *h*, plasma membranes of germinated spores at t_{85} and t_{200}, respectively.

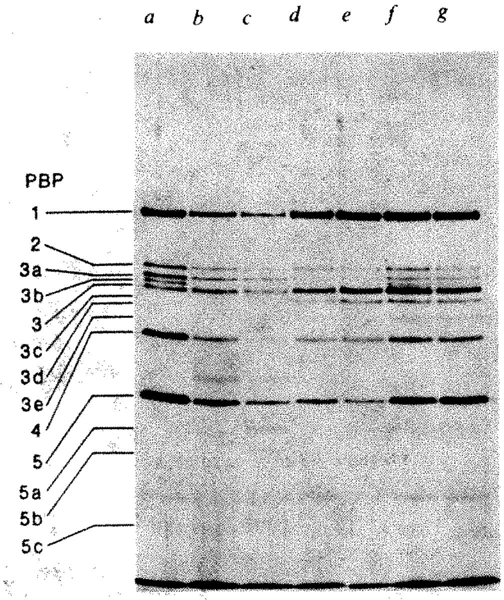

Fig. 2 Fluorogram of PBPs in the plasma membranes isolated from vegetative cells (*a*); sporulating cells at stage I (*b*) and stage II (*c*); and mother cells at stage III (*d*), stage IV (*e*), stage V (*f*) and stage VI (*g*). Mother cell plasma membranes, separated from the forespore membranes by differential centrifugation (see Fig. 1), and total cell membranes were prepared and assayed for PBPs as described in Fig. 1 legend.

During sporulation and, significantly, during cortex synthesis, dramatic changes in the number and level of PBPs were observed in the forespore membranes compared with the plasma membranes isolated from the corresponding mother cell (compare Fig. 1 *b–e* and Fig. 2*d–g*). Novel PBPs were designated PBP 1a (doublet), 1b, 2a, 3e, 5a, 5b and 5c (Figs 1, 2). PBPs 5b [MW ~ 41,000 (41 K)] and 5c (MW ~ 39 K) were not characterized further. These changes in PBPs are almost certainly sporulation-specific and not a response to nutrient limitation, as an asporogenic *B. megaterium* KM strain[20] grown in sporulation medium for the same time as an equivalent stage IV culture, did not show any significant changes in PBPs (data not shown).

Peptide maps of PBPs 1a and 1b, obtained by partial proteolysis with staphylococcal V8 protease, were identical to that of PBP 1 (Fig. 3), suggesting a common structural identity. Similarly, on appropriately exposed fluorographs it is apparent that PBP 3c may be related to PBP 1, PBP 2a to 2 and PBP 3b to 3a (data not shown). In contrast, PBPs 3e and 5a have unique peptide profiles suggesting that these proteins may be both novel and sporulation-specific as opposed to proteolytic fragments derived from higher molecular weight PBPs. Furthermore, limited proteolysis of PBP 5a with α-chymotrypsin (Fig. 3*c*) and papain (Fig. 3*d*) produced peptides distinct from those of PBP 5 and PBPs 3e, 1, 3 and 4 (Fig. 3*c*, *d*).

In preliminary experiments (J.T. and D.E., in preparation), PBP 5a showed D-alanine carboxypeptidase activity with a *p*H optimum similar to the vegetative cell membrane carboxypeptidase, which is attributed to PBP 5 (ref. 20). *p*-Chloromercuribenzoate (PCMB) inhibits the binding of penicillin only to PBPs 5, 5a and 3e (data not shown). PBP 3e was thus clearly distinguished from higher molecular weight PBPs by virtue of this sensitivity to PCMB, its lower affinity for benzylpenicillin (Table 1) and a completely distinct peptide profile (Fig. 3). It is likely, therefore, that PBP 3e represents a unique PSE active in the synthesis of spore-specific

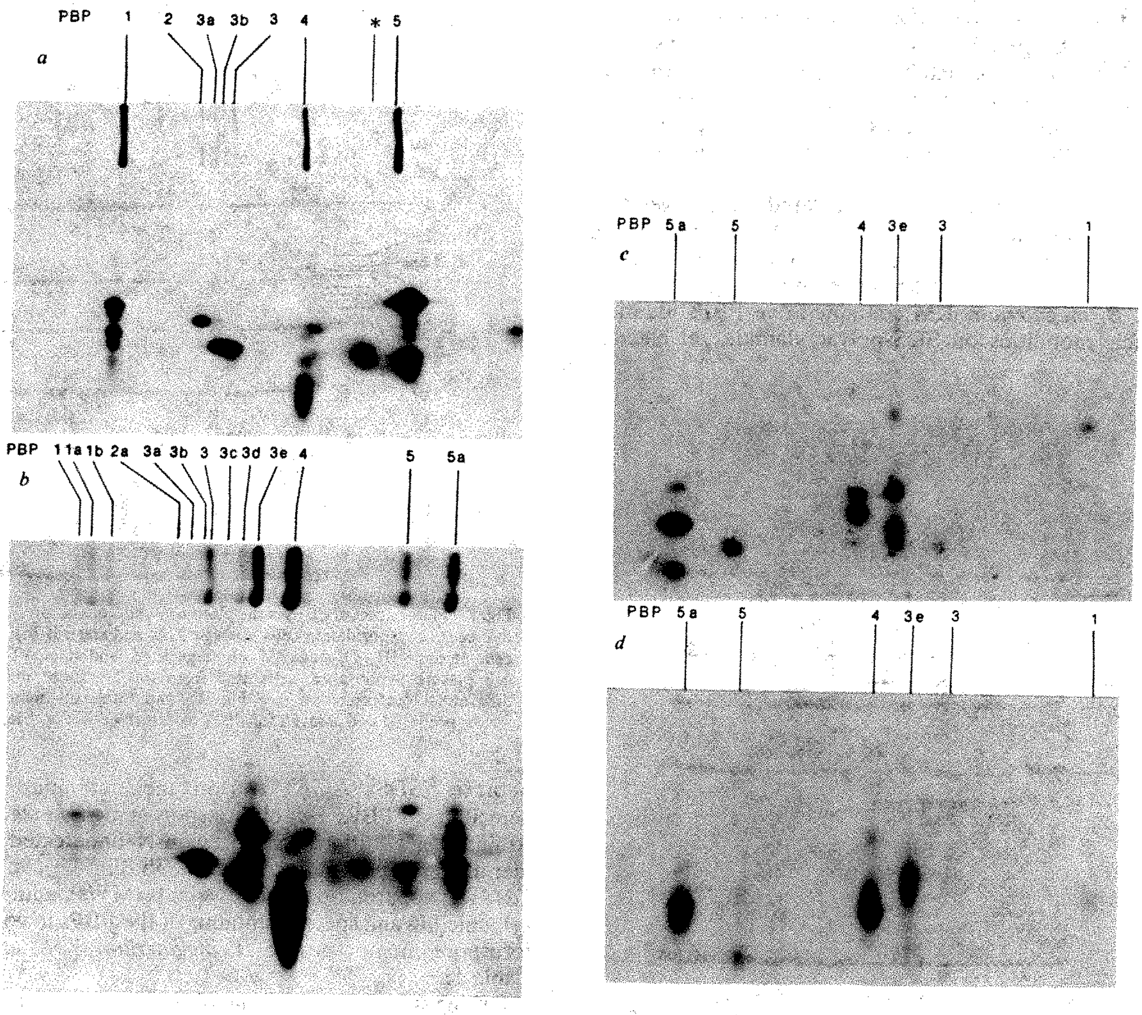

Fig. 3 Fluorograms of ^{3}H-benzylpenicillin-labelled peptides (obtained by partial proteolysis of PBPs with 100 μg of *Staphylococcus aureus* V8 protease) from vegetative plasma membranes (*a*) and stage VI forespore inner membranes (*b*). (*c*) and (*d*) show the peptide maps of PBPs 1, 3, 3e, 4, 5 and 5a obtained by partial proteolysis of stage IV forespore inner membranes with 125 μg α-chymotrypsin and 500 μg papain, respectively. The method used was based on that of Cleveland *et al.*[36], as modified by G. Stewart and D.J.E.[38]. Membrane protein (75–100 μg) was reacted with ^{3}H-benzylpenicillin (50 μg ml^{-1}) and separated by 10% SDS-PAGE as described in Fig. 1 legend. An 18% acrylamide gel was prepared with a 5-cm 6% acrylamide stacking gel. A 2-cm space was left above the stacking gel to accommodate the one-dimensional track which was overlayed with 0.5 ml of stacking gel containing the protease. Electrophoresis was as described previously[29] except that the current was switched off for 30 min just before the bromophenol blue entered the 18% gel. The gels were prepared for fluorography with sodium salicylate, as described by Chamberlain[37]. Duplicate tracks from the first dimension gel were fluorographed and shown in the figure as markers for each PBP in the peptide map. * The labelled peptide in this position is a result of nonspecific penicillin binding to a major membrane protein.

peptidoglycan. The second sporulation-specific protein, PBP 5a, may regulate the degree of cross-linkage (10-fold lower in spore peptidoglycan than in vegetative peptidoglycan[14,21]) and/or provide a suitable tetrapeptide substrate for the transpeptidase synthesizing spherical/ellipsoidal peptidoglycan at stage IV. PBP 5a may thus be analogous to PBP 5 in rod-shaped *E. coli* which, when overproduced, results in spherical growth[22].

In addition to the characteristic differences observed at stage V between the inner forespore membrane (ifm) (Fig. 1*d*) and the mother cell membrane (Fig. 1*f*), the outer forespore membrane (ofm) at this time is devoid of PBPs 1, 1a, 1b, 2, 2a, and 3a–3d and contains only 30% of the total forespore penicillin-binding capacity (data not shown). This suggests that the forespore compartment is directing the synthesis of novel PBPs into the inner forespore membrane and may indicate a role for the forespore cytoplasm and inner membrane in cortex assembly. A similar role was previously suggested by Frehel and Ryter[15] on the basis of their autoradiographic studies.

The re-establishment of the vegetative rod shape by a combination of swelling (t_0–t_{45}) and elongation (t_{45} onwards) during germination, was accompanied by a return to a PBP profile resembling that of the vegetative cell. This was achieved by a specific decrease in PBP 4 from t_{60} and the reappearance of PBP 1 from t_{45} as demonstrated by the PBP profile of cells at t_{85} (Fig. 1*g*). These data suggest that PBP 4 functions specifically in the synthesis of spherical peptidoglycan during t_0–t_{60} while PBP 1 may be concerned with polymer synthesis during elongation (a fuller characterization of germination-associated changes in PBPs will be presented elsewhere). The latter proposal is consistent with that of Reynolds *et al.*[23], who have suggested that PBP 1 is the major transpeptidase of *B. megaterium* KM. Hence, the substantial reduction in PBP 1 and the appearance of its proteolytic fragments, PBPs 1a and 1b, during sporulation may indicate a control mechanism that suppresses vegetative cell elongation in the stage III forespore as a prerequisite for the synthesis of spherical/ellipsoidal peptidoglycan. The role specified here for PBP 4 in the synthesis of spherical-shaped peptidoglycan is consistent with the results of Taku *et al.*[24]. They purified a PBP (MW ~ 61 K) from *B. megaterium* 899 having transglycosylase activity *in vitro* but with no demonstrable transpeptidase activity, which probably corresponds to PBP 4 of *B. megaterium* KM. Because PBPs 1a, 1b and 3 of *E. coli* are probably the transpeptidase/transglycosylase enzymes responsible for lateral wall and septum peptidoglycan synthesis respectively[3–6], Taku *et al.*[24] have suggested analogous enzyme activities and roles in cell growth of *B. megaterium* for PBPs 1 and 4.

The present paper provides the first experimental support for the hypothesis that PBPs/PSEs have central and distinct roles in the morphogenesis of Gram-positive rod-shaped bac-

Table 1 PBPs of the membranes from vegetative and sporulating cells and dormant and germinated spores of *B. megaterium* KM

			Specific levels of PBPs (no. of PBP molecules per 10^{-15} g of protein).									
							Membranes					
PBP	Apparent MW (K)	Benzylpenicillin affinity (μg ml^{-1})	Exp	I	II	III (ofm+ifm)	IV (ofm+ifm)	V (ifm)	VI (ifm)	DS (im)	GS t_{85}	GS t_{200}
1	115	0.04	5.0	1.3	0.8	0.8	0.1	0.1	0.1	+	1.0	3.6
1a	112	0.04	–	–	–	+	+	0.4	0.7	0.8	0.1	+
1b	107	0.04	–	–	–	+	+	0.4	1.1	1.2	0.1	+
2	92.5	0.10	1.0	0.7	0.2	+	+	+	–	–	0.2	0.7
3a	87.5	0.1	1.7	0.7	0.4	+	+	+	0.1	0.2	0.1	1.1
3b	85.5	0.1	0.8	+	0.1	+	+	+	0.1	0.2	0.1	1.1
3	84	0.3	1.0	1.3	0.7	0.3	2.5	1.0	0.7	0.7	0.4	3.5
3c	79.5	0.04	+	+	+	0.1	0.1	0.1	0.2	0.2	0.1	+
3d	76	0.01	+	+	+	0.1	0.1	0.1	0.4	0.8	0.1	+
3e	73.5	0.5	–	–	0.2	3.0	6.6	3.5	0.4	–	–	–
4	67	0.3	2.9	1.0	0.3	2.1	4.1	6.4	9.0	11.3	1.2	5.3
5	50	2.0	4.2	3.0	1.2	1.7	1.7	1.3	2.0	1.0	2.7	5.1
5a	44.5	2.0	–	–	0.4	3.8	10.1	2.0	0.1	–	–	–

Membranes were prepared from exponentially growing vegetative cells (Exp), stage I and II sporulating cells, stage III, IV, V and VI forespores, dormant (DS) and germinated spores (GS, t_{85} and GS, t_{200}) as described in Fig. 1 legend. Stage III and IV forespore membranes were a mixture of the ofm and ifm; stage V and VI forespore membranes and dormant spore membranes were, respectively, the ifm and spore inner membrane (im) only. The values of the affinities for benzylpenicillin of PBPs 1, 2, 3, 4 and 5 were taken from Chase *et al.*[20]. The affinities of the other PBPs for benzylpenicillin were estimated as described previously[20] (PBP 2a (MW ~ 90 K) has an affinity of 0.1 μg ml^{-1}, data not given). Molecular weights were estimated by the relation of log MW to the R_f of ^{14}C-methylated protein standards (Amersham). PBPs were quantified by estimation of the amount of radioactivity associated with the appropriate gel slice after location by fluorography (see Fig. 1 legend). Gel slices were prepared for liquid scintillation counting by the method of Tishler and Epstein[26]. Slices were solubilized by incubation at 50 °C for 24 h with 0.3 ml (30%/100 vols) of hydrogen peroxide. Determination of radioactivity was as described previously[27]. The number of PBP molecules per 10^{-15} g protein was calculated from the specific activity of the label. – Denotes that the PBP was not detectable after prolonged exposure of the fluorogram and + denotes that the counts associated with the PBP were insufficient to permit quantitation.

teria in a manner analogous to those of *E. coli*[3–6,22,25]. It also establishes PBPs 3e and 5a as unique sporulation-specific membrane-bound enzymes. The differentiation of PBPs during *B. megaterium* KM development should provide an ideal system to study differential gene expression in two separate intracellular compartments of a prokaryote and the mechanisms by which these proteins determine bacterial cell shape.

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Elucidation by FAB–MS of the structure of a new cardioactive peptide from *Aplysia*

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The nervous system and gut of *Aplysia* and other gastropod molluscs contain several classes of cardioactive peptides[1]. In addition to their excitatory action on the heart, these peptides stimulate contractile activity in the gut[1], and modulate neurally induced contractions of somatic muscles[2]. This report describes the purification and sequence determination by a combination of microprotein chemistry and fast atom bombardment mass spectrometry FAB–MS[3,4] of one of these peptides. The sequenced peptide is a member of the small class of cardioactive peptides and was termed SCP$_B$. A peptide with identical properties to SCP$_B$ has been found in several identified central neurones which innervate the gut[5]. Here we report the structure of SCP$_B$ as a C-terminally blocked peptide

H-Met-Asn-Tyr-Leu-Ala-Phe-Pro-Arg-Met-NH$_2$

Native and synthetic SCP$_B$ have identical actions on molluscan muscle preparations with thresholds at around 10^{-10} M.

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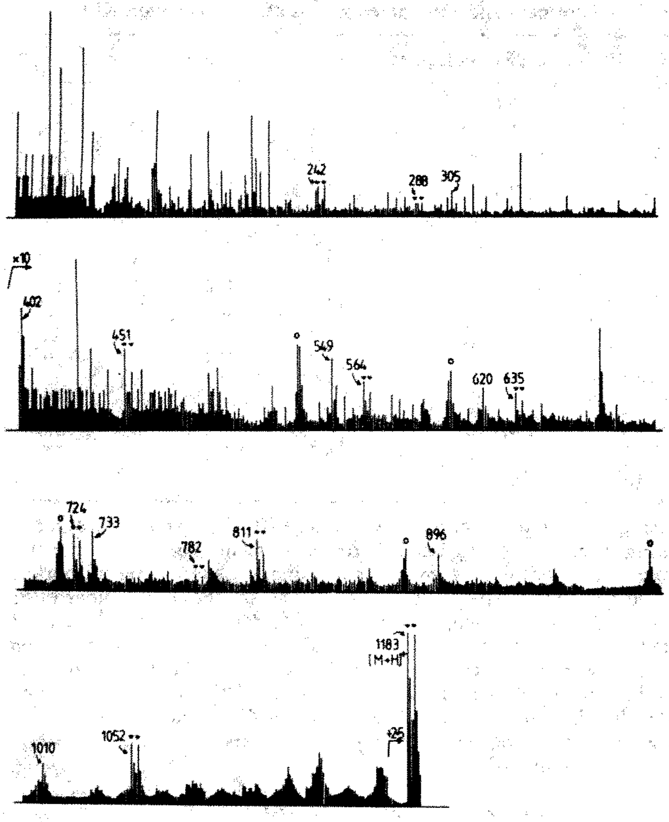

Fig. 1 Fast atom bombardment mass spectrum of the *N*-(1:1 acetyl: d_3-acetyl) derivative of SCP_B(1 μg). Ions arising from the N-terminus appear as 1:1 doublets, three mass units apart. The doublets and C-terminal singlets have been marked for clarity.

The central nervous systems from 2,500 *Aplysia brasiliana* were used as starting material. The homogenization, desalting and gel filtration steps were as previously described[6]. After each step, the active fractions were located by their inotropic action on an isolated snail heart assay[7]. The active fractions were applied to a cation-exchange column of SP-Sephadex (Pharmacia) at *p*H 6.0, and a linear gradient applied from 10 mM NH_4 acetate (*p*H 6.0) to 100 mM NH_4 acetate, 100 mM NH_4OH (*p*H 9.6). The cardioactive material eluted as a single peak which was further purified by HPLC. The sample was taken up in 0.1% trifluoracetic acid (TFA) and applied to a Bondapak C18 column (Waters). A reverse phase gradient was developed from the sample buffer to 0.07% TFA in acetonitrile. Two active peaks termed SCP_A and SCP_B were obtained (evidence from FAB–MS analyses suggests that the peak termed SCP_A contains the oxidized form of SCP_B which may account for its biological activity). Quantitative amino acid analysis suggested that SCP_B was a single peptide species with the following amino acid composition normalizing on Arg = 1:

$$\mathrm{Asx_{1.0}Ser_{0.2}Pro_{0.9}Gly_{0.3}Ala_{1.0}Met_{1.7}}$$
$$\mathrm{Leu_{1.2}Tyr_{0.8}Phe_{0.9}Arg_{1.0}}$$

The recovery from the final purification (1,000 animals) was 15–20%, with a yield of 10 nmol. Thus the original content of SCP_B in each nervous system (animal weight 400 g) was approximately 60 pmol.

After biological experiments and amino acid analyses 6 nmol were available for the mass spectrometric study. Each experiment was therefore restricted to 1.5 nmol (approximately 1 μg of SCP_B per analysis). The first sample was examined directly by FAB–MS as the free peptide, and gave a clear signal at 1,141, indicating a molecular weight for SCP_B of 1,140. This was in approximate agreement with that anticipated from amino acid analysis. Sequence ions in the spectrum were weak and the complexity did not allow an interpretation. A further 1 μg of SCP_B was treated with acetic anyhydride: d_6 acetic anhydride 1:1 v/v in methanol for 1 min at room temperature[8], followed by FAB–MS examination. The spectrum produced is shown in Fig. 1.

The appearance of a 1:1 signal (containing an acetyl group) at *m/e* 1,183 suggested that the N-terminus was not blocked, although abnormal acetylation of a very reactive guanidino group could not be eliminated at this stage. (Spinning-cup sequencer studies on the related SCP_A proved negative suggesting a possible blocked N-terminus). Importantly, the presence of weak 1:1 doublets throughout the spectrum now allowed a partial sequence of SCP_B to be built up by looking for meaningful mass differences based upon empirical FAB fragmentation mechanisms[4]. For example, the doublets at *m/e* 242, 288, 451, 564, 635, 724, 782 and 811 were spotted and have been marked on Fig. 1 for clarity. Not all of these led to readily interpretable mass differences but the signals at *m/e* 288, 451, 564, 635 and 782 gave a tentative partial sequence of

$$X^{288}\text{—Tyr-Leu-Ala-Phe—}Y$$

(where X and Y denote unknown portions of the molecule). Note that the signals at *m/e* 724 and 811 are not assigned and in fact were never used in the sequence assignment. They were assumed to be quasimolecular ions of contaminating peptides and this was later confirmed by their absence from the spectrum of the synthetic peptide.

At this stage, 1 μg of SCP_B was digested with trypsin *p*H 8.3 (1:50 enzyme to substrate, at 37 °C for 1 h) and the digest examined directly by FAB–MS. This experiment was crucial for the interpretation of the structure (Fig. 2). A new quasimolecular ion is seen at *m/e* 1,011, concomitant with the disappearance of the original signal at *m/e* 1,141. The new peptide giving rise to the 1,011 signal would be expected to contain a C-terminal arginine residue and its high mass placed that arginine towards the C-terminal end of the molecule. However the mass difference of 130 AMU lost on digestion does not correspond to any single amino acid or combination of amino acids. However, the mass difference is just one atomic mass number away from the mass of methionine. If we then postulate that SCP_B has a blocked amidated C-terminus, the tryptic digestion process would liberate a free carboxyl group from a previously amidated extended sequence, thus accounting for the one atomic mass unit and we therefore deduced that the SCP_B peptide contained a C-terminal-Arg-Met-NH_2 unit.

Because the 1:1 labelled signals (*m/e* 288 etc.), which appear to be derived from partial sequence -Tyr-Leu-Ala-Phe-, were not C-terminal ions extending N-terminally from the Arg-Met-amide sequence, it then followed that the label could not be on the arginine but was on the amino terminus and thus SCP_B was not N-terminally blocked.

Using this information we looked for C-terminal sequence ions in Fig. 1 and noted signals at *m/e* 549, 620, 733, 896 and

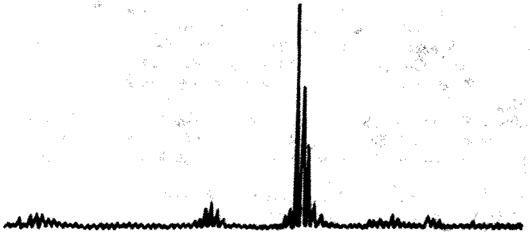

Fig. 2 Molecular ion region of the fast atom bombardment mass spectrum of the tryptic digest of SCP_B (1 μg), showing the presence of a quasimolecular ion $(M+H)^+$ at *m/z* 1,011.

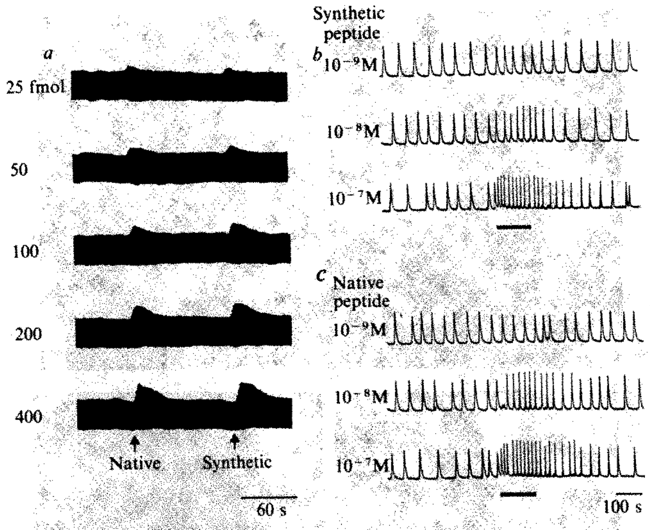

Fig. 3 A comparison of the biological activities of native and synthetic SCP$_B$s on two preparations. *a*, Pulse applications of 25–400 fmol of the peptides to an isolated snail heart. Native and synthetic peptides caused similar increases of the amplitude of heart beat contractions at each concentration. *b*, *c*, Superfusion of an isolated snail oesophagus with 10^{-9}–10^{-7} M synthetic SCP$_B$ (*b*), and native SCP$_B$ (*c*). The contractile activity of the oesophagus represents rearward directed peristalses which increase in frequency when either the synthetic or native SCP$_B$ is present in the bath (at horizontal bars).

1,010. These signals are all accompanied by satellites 15 AMU lower corresponding to the alkyl rather than the amine fragment with the charge on the C-terminus. This pattern often facilitates the identification of C-terminal sequence ions. By interpreting mass differences the sequence -Asn-Tyr-Leu-Ala has been generated.

The unknown unit 'X' on the N-terminal side of the tyrosine residue had a mass of 288 AMU, so by using C-terminal signals we have deduced that one component of this signal is asparagine. It now follows by mass difference that the N-terminal amino acid of SCP$_B$ is methionine, thus giving a structure H-Met-Asn-Tyr-Leu-Ala-.

Combining these data with the N-terminal assignment and trypsin experiment now extends the sequence to

H-Met-Asn-Tyr-Leu-Ala-Phe[···]Arg-Met NH_2

Using similar logic to that above, on any of the C-terminal signals, for example *m/e* 549, and subtracting the masses of Phe, Arg and Met amide, we conclude that the bracketed gap in the sequence above has a mass difference of 97 AMU which corresponds to proline. This assignment is confirmed by the observation of a signal at *m/e* 402 corresponding to the C-terminal signal Pro-Arg-Met-amide. Note that there is no satellite 15 AMU away, as would be expected since proline has a tertiary nitrogen, and cannot fragment to give an alkyl unit.

A combination of all the above analyses gives the structure of SCP$_B$ as the C-terminally blocked nonapeptide

H-Met-Asn-Tyr-Leu-Ala-Phe-Pro-Arg-Met-NH_2

The peptide was synthesised via *t*-butyloxycarbonyl (Boc) ester intermediates[9] using *N,N*-dicyclohexylcarbodiimide-1-hydroxybenzotriazole coupling[10]. The amide group was introduced at the Boc-Pro-Arg-Met methyl ester stage by treatment with methanolic ammonia. The protected nonapeptide was purified by countercurrent distribution and deprotected in 90% TFA. Reverse-phase HPLC showed that the synthetic peptide was 95–97% pure, and that it precisely co-eluted with the native peptide.

The biological activities of the native and synthetic peptide were compared on two types of preparation; isolated heart and oesophagus from snails. The isolated snail heart is the most quantitative of the available assays for SCP$_B$ but responds in similar fashion to the application of other substances such as other neuropeptides and serotonin[5]. In contrast, the isolated snail oesophagus is less quantitative but responds in a qualitatively different fashion to SCP$_B$, other neuropeptides and serotonin[1]. Native and synthetic peptides were quantified by amino acid analysis and applied in known amounts to the heart (Fig. 3*a*) and oesophagus (Fig. 3*b*). These results demonstrate that the active and synthetic peptides are quantitatively and qualitatively identical. We have also carried out some preliminary experiments which indicate that synthetic SCP$_B$ also excites the isolated *Aplysia* heart (threshold 10^{-11} M). Together with the FAB-MS analysis of synthetic SCP$_B$ these data confirm that the presented sequence is correct.

It is interesting to note that (1) our sequence terminates with several hydrophobic residues, (2) the penultimate residue is arginine, and (3) the C-terminus is amidated. This is strikingly similar to another molluscan cardioactive peptide, Phe-Met-Arg-Phe-amide[11]. Our work indicates that a new peptide class may exist, the key unit for biological activity being

Phe-A-Arg-B-amide

(where A and B are also hydrophobic amino acids), and probes designed with such a sequence may be valuable in the search for related peptides. Recent reports have suggested that there may be considerable conservation of neuropeptides between invertebrates and mammals, and there are a number of interesting cases where the original elucidation of a peptide class was carried out in an invertebrate. Examples of this include eledoisin (a tachykinin) from octopus[12], FRMF-amide from clam[13] and head-activating peptide from *Hydra*[14]. It will therefore be important to ascertain if peptides related to SCP$_B$ are present in other animals including mammals.

The precise physiological role of SCP$_B$ has not been determined in any gastropod mollusc, but it appears likely that it is involved in the control of gut activity[1,5]. In addition its marked potency in exciting many different muscle preparations suggests that it may be involved in a more general arousal in these animals.

Finally, we note that mass spectrometry, whilst previously essential in the elucidation of such structures as the enkephalins[15], leukotriene D[16] and adipokinetic hormone[17], has now improved in sensitivity by at least an order of magnitude. Although FAB-MS rarely gives complete sequence information at the 1 nanomole level, this paper demonstrates how a complete unknown sequence can be generated by judicious use of enzyme digests and micro-scale derivatization.

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Lectin binding reveals a synapse-specific carbohydrate in skeletal muscle

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Molecules concentrated at synapses are of interest because they may have roles in synaptic development or function. Several 'synapse-specific' proteins have been identified at the skeletal neuromuscular junction; acetylcholine (ACh) receptors[1], acetylcholinesterase[2], some components of the basal lamina[3] and a number of cytoskeletal elements[4,5], are all present at far higher concentrations at synaptic sites than elsewhere on the muscle fibre surface. As carbohydrates are thought to be involved in neuronal recognition, adhesion and synaptogenesis[6-12], we have asked whether any saccharide moieties are similarly concentrated at synaptic sites on muscle fibres. Lectins are proteins that bind to carbohydrates; each has its own sugar specificity[13,14]. Fluorescent and other derivatives of lectins have been used as histochemical stains to study the nature and distribution of carbohydrates on many cell types[13-16] including neurones[6,7,17-19] and muscle fibres[16,20]. We report here that the lectin from the legume *Dolichos biflorus*, DBA, selectively stains neuromuscular junctions in several vertebrate species. This result demonstrates the existence of a synapse-specific carbohydrate.

Cryostat sections of unfixed skeletal muscle were incubated with fluorescein-conjugated lectins and viewed by fluorescence optics. Fluorescein–DBA stained only a few discrete spots on the surface of rat muscle fibres (Fig. 1*a*). Double staining with rhodamine-α-bungarotoxin, which binds to ACh receptors in the postsynaptic membrane[1,3-5], showed that the DBA-stained patches were synaptic sites (Fig. 1*b*,*c*); no other structures identifiable in cross-sections were stained by this lectin. Fluorescein–DBA also stained neuromuscular junctions specifically and intensely in cryostat sections of rabbit, hamster and chick muscle; frog (*Rana pipiens*) neuromuscular junctions were faintly, but selectively stained. Thus, DBA recognizes molecules that are highly concentrated or especially accessible at the neuromuscular junction.

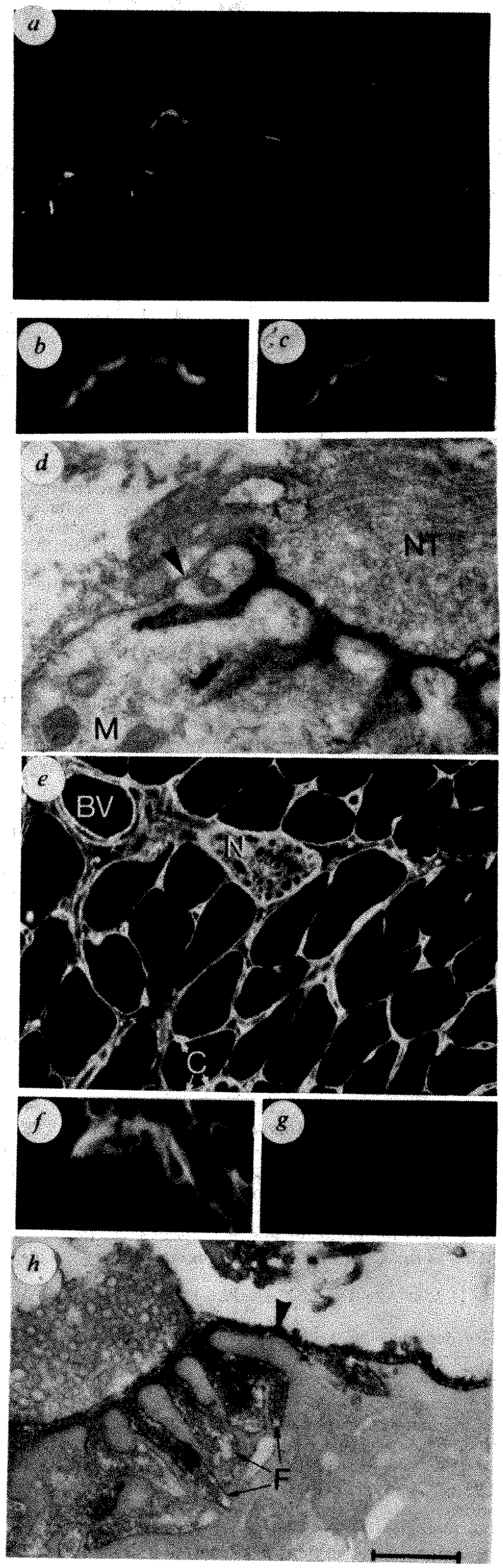

Fig. 1 *a–c*, Staining of rat neuromuscular junctions by fluorescein–DBA. Cross-sections of frozen, unfixed rat diaphragm were incubated for 30 min with a mixture of fluorescein–DBA (E-Y Laboratories) and rhodamine-α-bungarotoxin, then photographed with appropriate filters to show fluorescein (*a*, *b*) or rhodamine (*c*). Rhodamine–bungarotoxin binds to ACh receptors in the postsynaptic membrane and thus marks synaptic sites[3-5]. *b* and *c* show the same field; the coincidence of fluorescein and rhodamine demonstrates that DBA stains synaptic sites. Similar results were obtained with fluorescein–DBA from Polysciences, and with biotin–DBA plus fluorescein–avidin from Vector Laboratories. *d*, Electron micrograph of a neuromuscular junction from a rat intercostal muscle incubated with peroxidase–DBA, then washed, fixed and stained for peroxidase (see ref. 27 for details). Reaction product is present in the synaptic cleft between the nerve terminal (NT) and muscle fibre (M), and in junctional folds (F; see *h*), but not on extrasynaptic portions of the muscle fibre surface (arrowhead). No staining was detectable when muscles were incubated without lectin. *e–h*, Sections prepared as in *a–d*, but stained with *Ricinus communis* I lectin. This lectin stains both synaptic and extrasynaptic portions of the muscle fibre surface, as well as surfaces of blood vessels (BV), capillaries (C), and axons and perineural sheaths of intramuscular nerve trunks (N). Scale bar, 50 μm in *a* and *e*; 10 μm in *b*, *c*, *f* and *g*; 0.5 μm in *d*; and 0.4 μm in *h*.

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DBA is a lectin that recognizes N-acetylgalactosaminyl (GalNAc) termini of oligosaccharide chains[13,21]. Three results provide evidence that the staining of rat neuromuscular junctions by fluorescein–DBA represents binding of the lectin to a synapse-specific carbohydrate that contains or resembles Gal-NAc. First, staining was abolished by addition of 20 mM Gal-NAc to the incubation medium (Fig. 2*a*) but persisted in the presence of 100 mM, D-glucose, D-galactose, N-acetylglucosamine or α-methylmannoside. Thus DBA binds to synapses via its GalNAc-binding site. Second, two other lectins that recognize GalNAc residues, *Glycine max* (soybean) lectin and *Vicia villosa* lectin, stained synaptic sites intensely. These two lectins also stained the extrasynaptic muscle fibre surface faintly, possibly because they are less discriminating than DBA in their preference for GalNAc over other sugars[21,22]. Finally, lectins that recognize three other sugars do not distinguish synaptic from extrasynaptic regions; concanavalin A, wheatgerm agglutinin, and *Ricinus communis* I lectin (staining blocked by α-methylmannoside, N-acetylglucosamine and D-galactose, respectively) all stained both synaptic and extrasynaptic portions of the muscle fibre surface as well as the surfaces of blood vessels, capillaries, myelinated axons and intramuscular nerve sheaths (Fig. 1*e*–*h*). This result shows that specific staining of neuromuscular junctions by DBA is not due to an overall disproportion of carbohydrate at synaptic sites.

Further histochemical studies of rat muscle elucidated the localization of the synaptic component(s) to which DBA binds. Electron microscopy of muscle stained with peroxidase–DBA revealed reaction product in the synaptic cleft and in junctional folds but not elsewhere on the muscle fibre surface (Fig. 1*d*), confirming that this lectin binds selectively to synaptic sites. As muscles were incubated with peroxidase–DBA while alive, then washed, fixed and stained[3], at least some DBA receptors must be external to the protein-impermeable plasma membrane. Staining persisted without any apparent diminution in denervated muscle (Fig. 2*b*), demonstrating that DBA receptors are not confined to the nerve terminal, but are present on the muscle fibre surface. DBA also stained synaptic sites after muscle was subjected to either of two treatments that remove cytoplasm and plasma membrane but not the basal lamina, that is; damage-induced degeneration and phagocytosis of muscle fibres (Fig. 2*c*) or salt and detergent extraction of sliced muscle[3]. This shows that some DBA receptors are associated with the basal lamina of the synaptic cleft; it does not, however, exclude the possibility that DBA also binds to some components of the plasma membrane. (Some receptors for concanavalin A, wheatgerm agglutinin and *Ricinus communis* I lectin also survived muscle damage and thus are associated with basement membrane in muscle, as they are in kidney[23].)

The identity and function of the DBA receptor(s) we have stained are unknown. GalNAc-binding lectins recognize glycoproteins, glycolipids and glycosaminoglycans[13,21,24], any or all of which might be concentrated at synaptic sites. Among many possible roles for the DBA-binding carbohydrates are targeting of molecules to synaptic sites[12], adhesion of nerve and muscle[6], and recognition of synaptic sites by nerve terminals[25]. An intriguing idea is that there is an endogenous lectin[8,24] at the synapse which, in concert with the DBA receptor, plays some part in the formation or function of the neuromuscular junction.

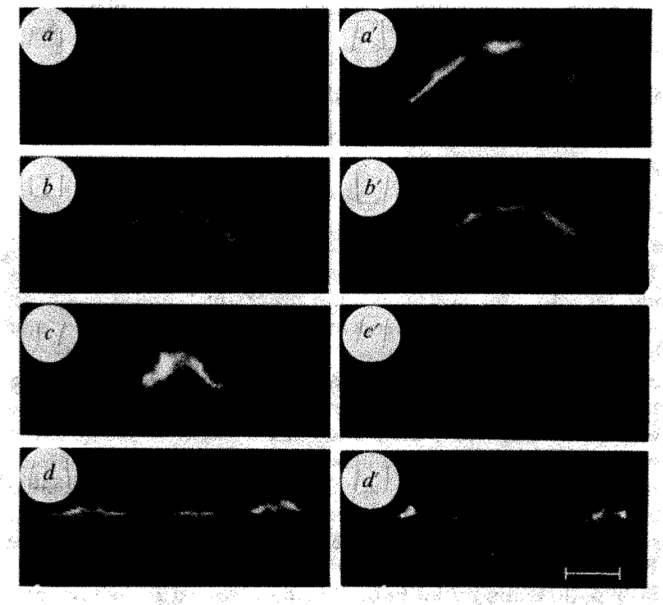

Fig. 2 Characteristics of DBA receptors in muscle. Muscles were doubly-labelled with fluorescein–DBA (or biotin–DBA followed by fluorescein–avidin) and rhodamine–bungarotoxin, as described in Fig. 1 legend. Each pair of micrographs shows the same field photographed with fluorescein- (*a*–*d*) and rhodamine- (*a'*–*d'*) selective filters. *a*, *a'*, Cryostat section of normal rate diaphragm stained in the presence of 20 mM GalNAc. The hapten sugar has blocked staining of the synaptic site by DBA; compare with Fig. 1*b*, *c*. *b*, *b'*, Cryostat section of rat diaphragm denervated 2 weeks earlier. Nerve terminals have degenerated and been removed, but DBA still stains synaptic sites. *c*, *c'*, Cryostat section of rat intercostal muscle that had been damaged[3] and denervated 3 days earlier. Nerve and muscle membranes have degenerated, and bungarotoxin-binding sites have disappeared, but basal lamina sheaths of muscle fibres survive (see refs 3, 27 for details); DBA stains synaptic sites on the sheaths. In other experiments, such DBA-stained patches were positively identified as synaptic sites by double staining with an antibody to a synapse-specific component of muscle fibre basal lamina[3]. *d*, *d'*, Myotube from a chick nerve-muscle co-culture prepared as described by Fischbach[28] (provided by Drs L. Role and G. Fishbach). DBA binds to ACh receptor-rich patches on the myotube surface. Scale bar, 10 μm.

These possibilities are best tested *in vitro*. In initial studies, we have found that fluorescein–DBA binds selectively (although not exclusively) to ACh receptor-rich regions of the myotube surface in chick nerve–muscle co-cultures (Fig. 2*d*); some of these patches are synaptic sites[26].

Thus, we have shown that the plant lectin DBA binds specifically to synaptic sites on the surface of skeletal muscle fibres; to our knowledge, this is the first demonstration of a carbohydrate moiety specifically localized at a synapse in any tissue. DBA should be a useful tool with which to study the identity, metabolism and function of this carbohydrate. In addition, DBA can serve as a simple light and electron microscopic stain for neuromuscular junctions.

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Sex differences in rat brain oestrogen and progestin receptors

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The brains of both male and female rats possess specific cytoplasmic receptors for oestrogens and progestins[1], and it has been suggested that sex differences in the neuroendocrine and behavioural responses to these hormones result from differences in the number of steroid receptors in specific locations in the brain[2]. Although there have been numerous comparisons of steroid receptors in male and female brains[3–9], the question of whether there are sex differences in these receptor systems remains unresolved, due in part to the inability of available biochemical techniques to achieve sufficiently high anatomical resolution. We have overcome this problem by applying the Palkovits punch-out technique[10] to measure steroid receptors in hypothalamic nuclei known to be involved in behavioural and neuroendocrine control. We report here that castrated male rats have fewer oestrogen and progestin receptors in certain hypothalamic nuclei than females. This suggests that the relative insensitivity of male rats to feminine actions of gonadal steroids might be due in part to a lack of receptors for these steroids in highly localized brain regions.

Hypothalamic nuclei were removed from frozen, 300-μm thick brain sections, using hollow stainless steel cannulas, 500 or 1,000 μm in diameter. We pooled tissue from three ovariectomized or castrated rats (CD-1 strain, Charles River Biological Laboratories; 150–180 g) for an assay. For measurement of progestin receptors, all rats received subcutaneous (s.c.) injections of 10 μg oestradiol benzoate (OB) once a day for 3 days before killing; this treatment induces hypothalamic progestin receptors to high levels[11,12]. Rats were perfused through the heart with 10% ice-cold dimethyl sulphoxide to protect steroid receptors against loss from freezing[13]. We removed from the brain sections portions of the bed nucleus of the stria terminalis, the medial preoptic nucleus, the periventricular region of the preoptic area and of the anterior hypothalamic nucleus, the ventromedial nucleus and the arcuate nucleus. These six nuclei and their subregions were examined because they have the highest concentrations of gonadal steroid receptors in the brain[14,15], and have been extensively implicated in the behavioural and neuroendocrine actions of steroids[16]. The number of oestrogen and progestin receptors was measured in cytoplasmic fractions of the nuclei by the specific binding of ^{3}H-OE$_2$ (115 Ci mmol^{-1}, NEN) and ^{3}H-R5020, a potent synthetic progestin (87 Ci mmol^{-1}, NEN). Cytosol protein (8–50 μg) was incubated with 1 nM ^{3}H-OE$_2$ or 0.4 nM ^{3}H-R5020 at 4 °C for 4 h. For each nucleus, the amount of protein in the incubate was the same for males and females (Table 1). Non-specific binding was measured by co-incubation with 1 μM moxestrol or 0.1 μM R5020, respectively. The ^{3}H-steroid bound to receptors was separated from free ^{3}H-steroid by gel filtration on Sephadex LH-20 columns[11–15]. The amount of protein in the incubate was determined by the method of Bradford[17]. The levels of oestrogen and progestin receptors in brain samples were expressed as steroid specifically bound per mg protein.

In preliminary studies, we observed a qualitatively similar distribution of gonadal steroid receptors in male and female brains. The highest levels of both oestrogen and progestin receptors in either sex were found in the periventricular region of the preoptic area, with substantial levels of binding also observed in the arcuate, medial preoptic and ventromedial hypothalamic nuclei, and in the periventricular region of the anterior hypothalamic nucleus. The bed nucleus of the stria terminalis showed high oestrogen binding, but low levels of progestin receptors. We observed moderate amounts of gonadal steroid receptors in the anterior hypothalamic nucleus, the dorsomedial nucleus of the hypothalamus, and the medial nucleus of the amygdala. Both males and females had low levels of receptors in the lateral preoptic nucleus, the nuclei of the septum and the diagonal band of Broca. We also observed similar, low levels of uninduced progestin receptors in males and females.

Although the general receptor distribution was the same for males and females, there were substantial quantitative sex differences in the receptor content of specific nuclei (Fig. 1). The medial preoptic nucleus, which may be the target region for oestrogen-induced control of gonadotropin release[16], had less than half the level of oestrogen receptors in males as in females (7×10^{-15} as opposed to 17×10^{-5} m (moles) per mg protein). There was a large sex difference in the progestin receptor content of the ventromedial nucleus, with oestrogen-treated male rats having less than 30% of the receptor levels of oestrogen-treated females (8 as opposed to 25×10^{-15} mol

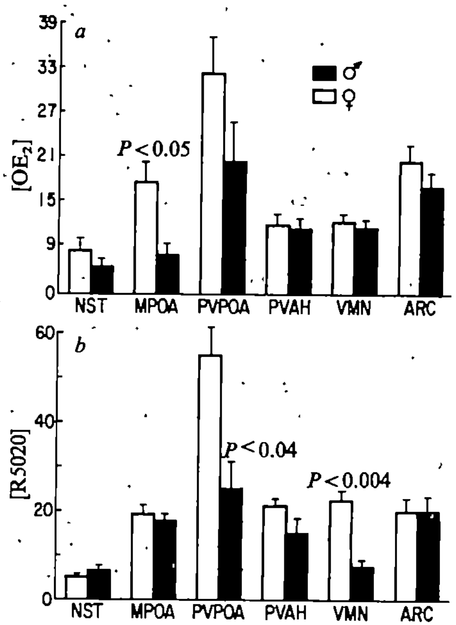

Fig. 1 Oestrogen and progestin receptor levels in hypothalamic nuclei of male and female rats. Rats were gonadectomized at least 1 week and not more than 3 weeks before assays were performed. Males and females were assayed together and were matched for age and length of time after gonadectomy. Steroid receptor levels were measured in hypothalamic nuclei by the specific binding to cytosol fractions of ^{3}H-oestradiol (*a*) and ^{3}H-R5020, a synthetic progestin (*b*). Tissue from hypothalamic nuclei was homogenized in 240 μl of TEGD buffer (10 mM Tris-HCl, 5 mM EDTA, 10% glycerol and 1 mM dithiothreitol *p*H 7.4). The cytosol fraction of the homogenate was obtained by centrifugation in a Beckman Airfuge at 100,000*g* for 20 min. 100 μl of cytosol were added to 50 μl of TEGD buffer containing ^{3}H-steroid plus unlabelled competitors. A 100-μl aliquot of the incubate was applied to a Sephadex LH-20 column for gel filtration. The column was eluted with 400 μl TEG buffer (TEGD buffer without dithiothreitol) 30 min after the sample was applied. The radioactivity in the eluate was measured by liquid scintillation counting at 34% efficiency. The level of nonspecific binding corresponded to no more than 25% of the total binding. Progestin receptor measurements were made in rats given oestradiol benzoate. Possible sex differences in non-oestrogen-induced progestin receptors were not examined because most of the neural actions of progesterone in females require oestrogen priming, and either require or are strongly correlated with oestrogen-induced progestin receptors[3]. Values are expressed as concentration of steroid ($\times^{-15}$ mol) specifically bound per mg protein· $n=9$ for paired comparisons of oestrogen receptor levels in males and females; $n=6$ for paired comparisons of progestin reeptor levels. Significant sex differences were seen in oestrogen receptor levels in the medial preoptic area ($P=0.045$, 2-tailed paired *t* comparison), in progestin receptors in the periventricular preoptic region ($P=0.037$) and in the ventromedial nucleus ($P=0.003$). There were no other significant sex differences in receptor content ($P>0.120$ for all nuclei, 2-tailed paired *t*-test). NST, bed nucleus of the stria terminalis; MPOA, medial preoptic area; PVPOA, periventricular region of the preoptic area; PVAH, periventricular portion of the anterior hypothalamic nucleus; VMN, ventromedial ucleus; ARC, arcuate nucleus.

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Table 1 Location and protein content of hypothalamic nuclei

Region	Stereotaxic location	Protein (mg) Male	Protein (mg) Female
Bed nucleus stria terminalis	A7,190–6,290 μm	0.024±0.001	0.026±0.001
Medial preoptic nucleus	A7,190–6,590 μm	0.021±0.001	0.020±0.001
Periventricular preoptic nucleus	A7,190–6,590 μm	0.012±0.001	0.012±0.001
Periventricular anterior hypothalamic nucleus	A6,590–5,690 μm	0.018±0.002	0.017±0.001
Ventromedial nucleus	A4,890–3,690 μm	0.042±0.002	0.045±0.002
Arcuate nucleus	A4,890–3,690 μm	0.037±0.003	0.035±0.003

Nuclei were removed from frozen 300-μm thick sections of male and female rat brain, according to the procedure of Palkovits[10], using a 500 or 1,000 μm diameter stainless steel cannula. The medial preoptic and ventromedial nuclei were removed with bilateral 1,000 μm punches. The periventricular anterior hypothalamic nucleus was taken with a single 1,000 μm punch, placed over the dense area of ^{3}H-OE$_2$ binding described in the autoradiograms of Pfaff[16]. The bed nucleus of the stria terminalis was dissected with bilateral 500 μm punches above and below the decussation of the anterior commissure, and with bilateral 1,000 μm punches caudal to this. The periventricular preoptic region and the arcuate nucleus were removed with a small scalpel. The locations of nuclei are taken from the stereotaxic atlas of Konig and Klippel[21]. The protein values are the mean±s.e.m. of the amount of cytosol protein applied to Sephadex LH-20 columns for steroid receptor measurements (see text for details). Protein was measured by the method of Bradford[17]; $n = 15$ for both males and females.

per mg protein). The level of progestin receptor in the male ventromedial nucleus after OB treatment was comparable to unstimulated (non-OB-treated) levels in the female ventromedial nucleus[15]. The ventromedial nucleus of the hypothalamus may be the principal target site in rat brain for the activation of proceptive and receptive sexual behaviour by progesterone[3]. The periventricular region of the preoptic area also showed a significantly lower level of progestin receptor in males than in females (25 as opposed to 50×10^{-15} mol per mg protein). There were no other significant sex differences in oestrogen or progestin receptor content among the six nuclei examined (Fig. 1).

The sex differences in certain regions having a high content of oestrogen and progestin receptors could explain particular sex differences in sensitivities to gonadal steroids. The substantially lower level of oestrogen receptors in male medial preoptic nucleus might explain in part why luteinizing hormone (LH) release in male rats is not stimulated by oestrogen. The local implantation of oestrogen into the medial preoptic area will stimulate LH release, while ablation of the medial preoptic nucleus blocks the LH surge[16]. The very low level of progestin receptors in the ventromedial nucleus after oestrogen treatment may explain the inability of progesterone to produce proceptive behaviours and increase receptivity in oestrogen-primed males[3]. The implantation of nanogramme amounts of progesterone into the ventromedial nucleus will facilitate lordosis and activate sexual proceptivity in oestrogen-primed females[18]. The same small quantities of progesterone have no effect on sexual behaviour when applied to other hypothalamic nuclei. The effects of progesterone can also be blocked by the local application of a protein synthesis inhibitor to the ventromedial hypothalamus[19]. There is little information on the possible functions of the high level of progestin receptors in the periventricular preoptic area, thus the significance of the large sex difference in this region is unclear. It is possible that sex differences also exist in brain regions with a low to moderate amount of gonadal steroid receptors. These regions were not examined in the present study.

Sex differences in oestrogen and progestin receptors do not necessarily occur in the same nuclei (Fig. 1). In the medial preoptic area, the difference in oestrogen receptor content is not accompanied by a sex difference in progestin receptors: It may be that the subset of oestrogen-sensitive cells which make progestin receptors are not sexually dimorphic. Conversely, the sex differences in progestin receptors in the ventromedial and periventricular preoptic nuclei are not accompanied by a sex difference in oestrogen receptors; it is possible that the process of progestin receptor induction has been suppressed in these nuclei during sexual differentiation.

In previous studies on whole regions of the hypothalamus and preoptic area[3,4], we found no sex differences in cytoplasmic oestrogen and progestin receptors. The amount of steroid receptor in an individual nucleus is a small part of the total amount of receptor in a hypothalamic tissue block, thus it is not surprising that sex differences were not detected in these studies. Our measurements of cytoplasmic receptor levels do not exclude the possibility that sex differences also exist in the translocation of gonadal steroid receptors to cell nuclei. There are several reports of sex differences apparent in whole hypothalamic blocks of rat brain, when the nuclear retention of ^{3}H-oestradiol is measured[5–7], although other laboratories have found similar levels of nuclear uptake in males and females[8,9]. The measurement of cell nuclear receptor levels for gonadal steroids in microdissected brain regions would clarify the existence of sex differences in nuclear uptake. Note also that in order to minimize binding to low-affinity progestin sites[12], we used a concentration of ^{3}H-R5020 that occupies only 70–80% of the high-affinity component of the binding. Until we are able to perform binding isotherms on the limited amount of receptor in a punch-out sample, it remains possible, although unlikely, that the rather large male–female differences in ^{3}H-R5020 binding represent differences in affinity rather than differences in concentration.

It is possible that regionally localized sex differences in gonadal steroid receptors exist in the brains of many species. Using quantitative autoradiography, Arnold and Saltiel[20] have found sex differences in the nuclear uptake of androgens in the song control nuclei of zebra finch brain. Their results and those presented here indicate that sex differences in neural receptors for gonadal steroids could be a general consequence of sexual differentiation, and may even exist in steroid-concentrating regions of human brain.

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A circulating inhibitor of $(Na^+ + K^+)$ATPase associated with essential hypertension

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The aetiology of essential hypertension, a disease prevalent in cultured societies, is unknown. However, much evidence suggests that abnormal sodium metabolism has a critical role—this has led to the hypothesis that an increase in the circulating concentration of an inhibitor of $(Na^+ + K^+)$ATPase is responsible for the increased peripheral vascular resistance in essential hypertension[1]. Evidence for relatively high levels of a Na^+ pump inhibitor in essential hypertension has come from bioassay and cytochemical assays of plasma and urine from normotensive and hypertensive individuals[2,3]. There is also evidence for increased plasma levels of a Na^+ pump inhibitor in some animal models (for example, renal and deoxycorticosterone acetate (DOCA) models) of hypertension[4]. Nevertheless, direct biochemical determination of $(Na^+ + K^+)$ATPase inhibition by this substance has not yet been reported. We demonstrate here, with a kinetic $(Na^+ + K^+)$ATPase assay, a highly significant correlation between levels of a plasma inhibitor of $(Na^+ + K^+)$ATPase activity and mean arterial blood pressure (MAP) in normotensive and hypertensive individuals. These data provide evidence for the involvement of a circulating Na^+ pump inhibitor in the genesis of essential hypertension. Moreover, our assay methods may be useful for the isolation and characterization of this inhibitor.

We have tested the effect of deproteinized blood plasma samples on the activity of a partially purified canine kidney $(Na^+ + K^+)$ATPase. In our assay, the regeneration of enzymatically hydrolysed ATP is coupled to the oxidation of NADH so that the action of various inhibitors can be monitored by continuously recording the absorbance of NADH at 340 nm. As we are concerned here with the activity of a humoral inhibitor of $(Na^+ + K^+)$ATPase, we compared it with the behaviour of two well characterized inhibitors of the ATPase; Fig. 1*a* shows the features of inhibition by ouabain. This cardiotonic steroid had no significant effect on the reaction for the first 30 s, and thereafter caused a progressive, dose-dependent increase in inhibition[5]. Steady-state levels of inhibition were not achieved in the short time course presented. Similarly, the inhibition by vanadate (Fig. 1*b*) increased with time[6], but because of the much faster association rate constant for binding, an effect of vanadate was apparent during the first few seconds of the reaction, especially at higher concentrations. By measuring the steady-state levels of $(Na^+ + K^+)$ATPase activity in the presence of various vanadate concentrations, we estimate a K_i of 0.9 μM, in agreement with published values[7,8].

Figure 1*c* shows the kinetic behaviour of $(Na^+ + K^+)$ATPase assayed in the presence of a saline solution (control) and deproteinized plasma samples derived from one normotensive individual, and from a patient with essential hypertension. In each case, about 1.5 min were required to reach a steady state of enzyme activity, which then lasted for at least 10 min (not shown). The normotensive NT plasma produced (in this

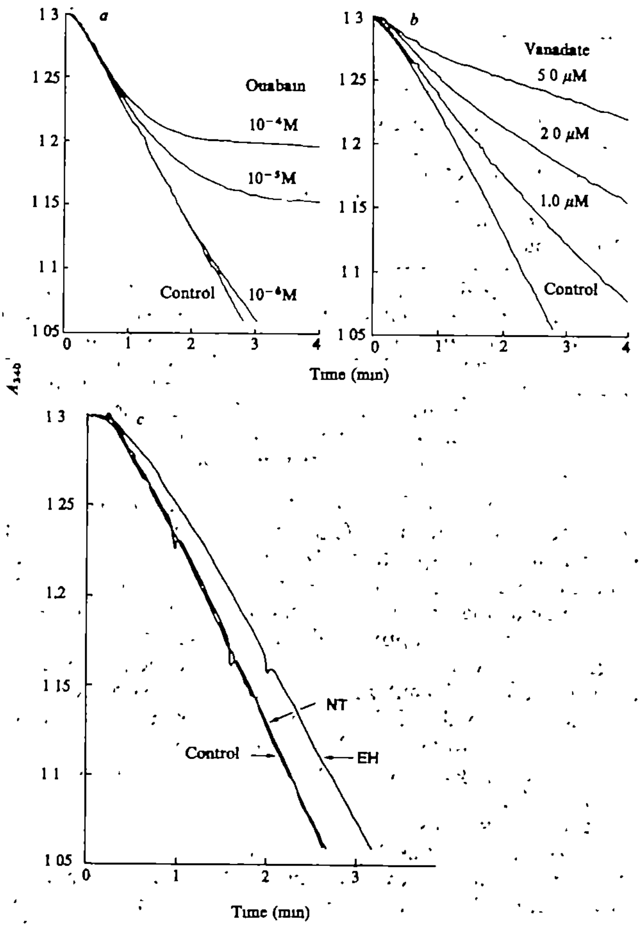

Fig. 1: Inhibition of $(Na^+ + K^+)$ATPase activity by ouabain (*a*), vanadate (*b*), and plasma samples (*c*). Canine kidney $(Na^+ + K^+)$ATPase was obtained from Sigma (specific activity 5–6 μmol per min per mg protein). This preparation also contained an ouabain-insensitive Mg^{2+}-ATPase activity which was less than 2% of the total steady-state ATPase activity. The ATPase assay consisted of 400 μl ATPase cocktail to which 580 μl of H_2O were added Final reaction conditions were (in 1.0 ml) as follows (in mM or appropriate units)· KCl 20; NaCl 100; $MgSO_4$ 4.5; EGTA 5; Na_2ATP 3; phosphoenolpyruvate tricyclohexylammonium (PEP), 1.2, NADH, 0.25; TES–Tris, 40, *p*H 7.4; lactate dehydrogenase (LDH), 1.2 U, pyruvate kinase (PK), 1.2 U. ATP, PEP, NADH, LDH and PK were from Boehringer Mannheim Biochemicals; both enzymes were obtained as glycerol suspensions. $(Na^+ + K^+)$ATPase was prepared for assay by suspension in 500 μM Na_4EDTA, 10 mM trimethylaminoethanesulphonic acid (TES)–Tris *p*H 7.4, at a protein concentration of 150 μg ml^{-1}. The resuspended enzyme was incubated at 25 °C for 3 h, and then stored on ice; the activity of the ATPase thus prepared does not change by more than 1% during the following 20 h on ice. The assay was initiated by rapid addition of 20 μl of ATPase suspension (3 μg protein) to 980 μl of reaction cocktail in a 1.0-ml cuvette at 37 °C, and the decrease in A_{340} due to NADH oxidation ($\varepsilon = 6.22 \times 10^3 \, l \, mol^{-1} \, cm^{-1}$) was continuously recorded, as illustrated in the examples shown here. *a*, Ouabain (1-μl aliquots of stock solutions prepared in dimethyl sulphoxide, DMSO) was pre-added to the reaction cocktail to give the final concentrations indicated. Controls contained appropriate quantities of DMSO. *b*, Vanadate, as Na_3VO_4, was freshly prepared in H_2O and similarly pre-added to the reaction. Neither ouabain nor vanadate, at the concentrations shown, had any significant effect on the efficacy of the regenerating system, as determined by inspection of rates of NADH oxidation in response to addition of 10–50 μM ADP. The activity of the regenerating system was found to be over 70-fold greater than that of the total ATPase activity in these conditions. *c*, Effect of two individual plasma samples on the ATPase activity. Conditions were similar to those for *a* and *b* except that the assay cocktail contained (in mM): $MgSO_4$, 9; and $CaCl_2$, 2. 500 μl of H_2O were replaced by an equivalent volume of deproteinized plasma (see Fig. 2 legend) obtained from a normotensive individual (NT) or a patient with essential hypertension (EH). The control contained, in addition to the assay cocktail, 500 μl of a solution of the following composition (in mM). KCl, 2.1; NaCl, 120; $NaHCO_3$, 26; $CaSO_4$, 2.5; K_2HPO_4, 1.2; $MgSO_4$, 1.2; EGTA, 3.4, EDTA, 2.8; and 800 μg ml^{-1} bovine serum albumin. The EDTA and EGTA were included in this solution to control for their presence as anticoagulants in the plasma samples; see Fig. 2 legend for details. All the curves shown were traced from original records. Per cent inhibition was calculated as:

$$100 - \left(\frac{\text{sample } \Delta A_{340} \text{min}^{-1}}{\text{control } \Delta A_{340} \text{min}^{-1}}\right) \times 100.$$

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example) no inhibition of activity compared with the control. In contrast, the hypertensive sample produced a 14% inhibition of steady-state activity which remained constant for the next 10 min of the reaction. This result demonstrates the presence of an increased level of an inhibitor of $(Na^+ + K^+)$ATPase in hypertensive plasma that has a rapid-onset of action in contrast to ouabain and vanadate.

This technique (see Fig. 1 legend) was used to assay plasma samples from 20 normotensive individuals and 26 patients with essential hypertension. A significant correlation between MAP and $(Na^+ + K^+)$ATPase inhibition was found when the hypertensive group alone was analysed ($r = 0.46, P < 0.01$); no significant correlation was found for the normotensive group. However, inclusion of the normotensives in the overall analysis (as illustrated) resulted in greater correlation coefficient and significance values ($r = 0.73, P < 0.0005$). Slightly smaller correlation coefficients ($r = 0.69$ and 0.68, respectively), but similar P values, were obtained when the data from blacks and whites were evaluated separately. The correlation was similar ($r = 0.77, P < 0.0005$) when diastolic pressure was used instead of MAP. There was no significant correlation between $(Na^+ + K^+)$ATPase inhibition and patient age, perhaps because we attempted to age-match normotensives and hypertensives. The absence of females from the hypertensive group precludes an evaluation of the role of sex. One of the previously diagnosed hypertensive patients was normotensive on the day of blood withdrawal (MAP = 91 mm Hg); plasma samples from this individual exhibited a low (<4%) $(Na^+ + K^+)$ATPase inhibition. We have not yet obtained samples from patients with secondary forms of hypertension, therefore we cannot comment on the applicability of this assay as a diagnostic tool to discriminate between essential hypertension and secondary hypertension.

The maximal inhibition observed in this study was 14%—this is an underestimation of the inhibition expected *in vivo* because the plasma samples were diluted twofold in our assay. It seems reasonable to conclude that the apparent inhibitory activity represents an effect on $(Na^+ + K^+)$ATPase *per se*, because the contribution of ouabain-insensitive Mg^{2+}-ATPase to the total steady-state hydrolysis of ATP is less than 2% in these conditions.

As our $(Na^+ + K^+)$ATPase preparation consisted of unsealed membrane sheets[9], we were unable to distinguish between an external and an internal site of action for the inhibitor. The data of Pamnani *et al.*[4] on experimental models of hypertension, and those of Poston *et al.*[3] on essential hypertension, indicate that the inhibitor acts at an external site, assuming (1) that the inhibitor does not cross the plasma membrane quickly, and (2) that our inhibitor is the same as theirs. Our kinetic data imply that the circulating inhibitor must bind much more rapidly than ouabain.

Figure 3 incorporates further data on red cell Na^+ levels and digoxin-like immunoreactivity in these plasma samples. The mean essential hypertensive MAP was more than two standard deviations above the mean normotensive MAP. Mean % inhibition of $(Na^+ + K^+)$ATPase was significantly elevated in the EH group, as was intracellular erythrocyte Na^+ content.

The plasma samples were tested for digoxin-like immunoreactivity, following the report of an endogenous digoxin-like material in the plasma of volume-expanded dogs[10] and hypertensive monkeys[11]. This substance has been postulated to be an endogenous ligand for the cardiac glycoside binding site of the Na^+ pump. The action of such a substance may therefore be related to inhibition of Na^+ transport and elevation of cellular Na^+ as described in essential hypertension[12,13]. However, as illustrated in Fig. 3, we were unable to demonstrate a significant elevation of digoxin-like immunoreactivity in the plasma samples that inhibited the $(Na^+ + K^+)$ATPase. Furthermore, there was no correlation between the two assays, which suggests that the $(Na^+ +$

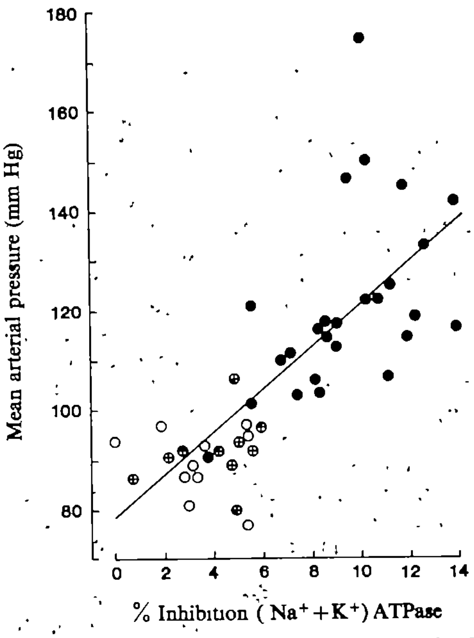

Fig. 2 Relationship between mean arterial blood pressure and inhibition of $(Na^+ + K^+)$ATPase by plasma samples from normotensive and hypertensive individuals; 20 normal subjects (age 34 ± 14 yr, range 21–66 yr) and 26 patients (age 49 ± 11 yr, range 32–67 yr) suffering from essential hypertension were studied. All the hypertensive subjects were males; 18 were black. Twelve of the normotensives were males, and six were black. None of the normotensives had known endocrine abnormalities, and none of the hypertensive patients showed any evidence of end-organ damage or serious intercurrent disease. All patients with essential hypertension had blood pressures higher than the 95th percentile for age and sex on at least three previous office visits, and had received no drugs for at least 2 weeks before the study. Thirty-six individuals, including all the patients with hypertension, were characterized endocrinologically using integrated hormone profiling based on the principle of continuous 6-h blood withdrawal[23,24]. The rationale for this collection protocol has been discussed elsewhere[23]. Plasma samples were collected continuously for 6-h periods using an indwelling non-thrombogenic catheter connected to a constant withdrawal pump. A sample (10 ml) was collected every 30 min into glass tubes containing (final concentrations) 2.8 mM EDTA, 3.4 mM EGTA and 2.6 mM glutathione on ice. Cells and plasma were separated by immediate centrifugation and the plasma was stored on ice. After the 6-h withdrawal, aliquots of the 30-min samples were combined to give a 6-h integrated pool. Plasma renin activity, aldosterone, cortisol, noradrenaline and adrenaline were measured as described elsewhere[23,24], no significant correlation was found between levels of these substances and blood pressure (data not shown). Systolic and diastolic blood pressures were measured at hourly intervals during blood collections, and the results were averaged. Mean arterial pressure was calculated as: MAP = 0.33 × pulse pressure + diastolic pressure. Plasma samples were analysed for Na^+ and K^+ (by flame photometry), inorganic phosphate[25] and protein content[26], and were stored at −20 °C. Plasma was subsequently deproteinized by acidification to pH 5.5 with 1.0 M HCl and boiled for 2 min. The resultant slurry was centrifuged at 30,000g for 30 min at 4 °C, and the clear supernatant removed for electrolyte and protein content determinations as before. The degree of protein removal was typically 99%, giving a final protein content of $800 \pm 180\ \mu g\ ml^{-1}$ in the supernatants. The deproteinized samples were then assayed for $(Na^+ + K^+)$ATPase inhibitory activity as described in Fig. 1*c* legend, and the remainder was lyophilized for use in the digoxin radioimmunoassay (RIA) (see Fig. 3). The order of assay samples was randomized to avoid bias. The average coefficient of variance for repeated estimations on the same samples was typically 1% or better. The final electrolyte composition of the assay cocktail varied because of the addition of plasma samples. Thus, the final assay conditions varied as follows. (in mM except as stated) K^+, 22–23; Na^+, 170–175; total Ca^{2+}, 3.25–4.5; free Ca^{2+}, $<10^{-7}$ M; total Mg^{2+}, 9.5–9.6; free Mg^{2+}, 2.1–2.8, total EGTA, 6.7; free EGTA, 2.2–100 μM; total EDTA, 1.4–2.1, free EDTA, $<10^{-8}$ M; total ATP, 3; free ATP, $<10^{-7}$ M; PO_4, 0.4–0.6. Surprisingly, we found that controlled variation of K^+ (20–30 mM), Na^+ (100–200 mM), free EGTA (1–500 μM), free Mg^{2+} (2–2.8 mM), and PO_4 (0.4–0.6 mM) gave no more than a 2% change in the steady-state activity of $(Na^+ + K^+)$ATPase. High concentrations ($>10^{-4}$ M) of free Ca^{2+} and ATP are both potent inhibitors of the ATPase but, due to the presence of excess EGTA and Mg^{2+}, respectively, they are reduced to non-interfering levels in our assay. In addition, the excess EGTA also serves to chelate other trace elements that may interfere with the assay. Given these and other considerations, we estimate that the cumulative error for each determination of ATPase activity was ~3%. This uncertainty and the variability of blood pressure could contribute to the scatter observed. ●, ⊕ Correspond to 6-h integrated samples collected from patients with essential hypertension and from normotensives, respectively. ○, Discrete single samples collected from normotensives. The regression line was fitted by the least-squares method; the correlation between MAP and % inhibition is highly significant ($r = 0.73, P < 0.0005$)

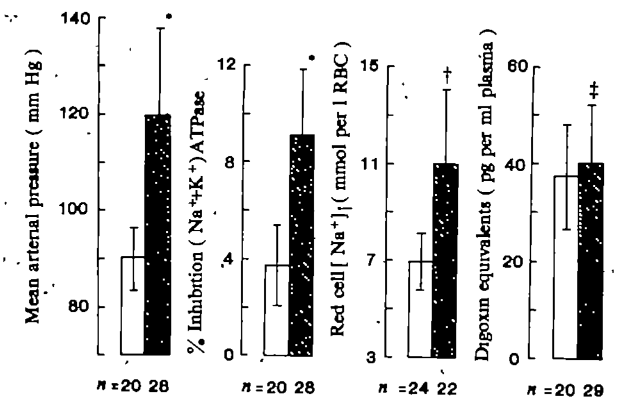

Fig. 3 MAP, % $(Na^+ + K^+)$ATPase inhibition, intracellular red blood cell (RBC) Na^+ content and plasma digoxin equivalents in normotensive (open bars) and hypertensive (cross-hatched bars) individuals. Determination of MAP and inhibition of $(Na^+ + K^+)$ATPase are described in Fig. 2 legend. Red cell Na^+ content was determined on freshly collected cells by atomic absorption spectroscopy after three washes of the cells in isotonic $MgCl_2$, determination of haematocrit and lysis of cells in deionized H_2O. Subsequent dilutions were performed in CsCl. For technical reasons, 34 of the red cell Na^+ determinations were performed on cells from individuals other than those tested for $(Na^+ + K^+)$ATPase inhibition and digoxin-like immunoreactivity However, all the blood samples were obtained from individuals in the same populations as those identified for study. Plasma digoxin equivalents were determined by digoxin RIA (NEN) essentially as described by Gruber *et al.*[10]. Deproteinized plasma samples were lyophilized and resuspended to 10× their original concentration in H_2O and assayed for their ability to compete with iodinated digoxin for a digoxin-specific antibody. Some samples were seeded with digoxin to ensure that the antibody was not affected by the increased electrolyte content of the assay. The data presented are corrected for concentration and dilution factors incurred during sample preparation, and are described as pg digoxin equivalents per ml plasma. None of the individuals had received cardiac glycosides. Values shown are mean±s.d. of the indicated number of samples. Significance of the differences in the means was determined by Student's *t*-test: $^*P < 0\ 0005$; $^\dagger P < 0.005$; ‡not significant ($P > 0.1$)

K^+)ATPase inhibitor we describe and the digoxin-like substance may not be related.

We have previously discussed the manner in which inhibition of Na^+ transport may lead to a rise in blood pressure[1,14,15]. Inhibition of Na^+ pumps in vasular tissue by ouabain results in increased resting tone[16] and enhanced reactivity to catecholamines[16]. As an increase in intracellular free Ca^{2+} is the immediate trigger for concentration in vascular smooth muscle[17], it seems reasonable to suppose that inhibition of Na^+ pumps leads to a net accumulation or redistribution of intracellular Ca^{2+}. This phenomenon may occur as a consequence of a Na^+–Ca^{2+} exchange mechanism at the vascular smooth muscle cell plasma membrane[1,14,15], whereby the increase in intracellular Na^+ by Na^+ pump inhibition leads to a net uptake of Ca^{2+} and, thus, to an increase in peripheral vascular resistance—the hallmark of the hypertensive process[18]. In addition, inhibition of Na^+ pumps in sympathetic nerve terminals may enhance catecholamine release[19] and reduce re-uptake[20], and thus contribute to the maintenance of elevated vascular smooth muscle tone[15].

The sequence of events leading to the elevated levels of $(Na^+ + K^+)$ATPase inhibitor in the plasma may be the result of a congenital (or, in some types of secondary hypertension, acquired) defect in the renal natriuretic capability in individuals prone to develop hypertension[21]. Excessive Na^+ ingestion by these individuals would result in Na^+ and water retention and the tendency towards expansion of the extracellular fluid volume unless adequate compensatory physiological mechanisms are brought into play. Our data support the view that one such compensatory mechanism may be increased secretion of an inhibitor of $(Na^+ + K^+)$ATPase that enhances Na^+ excretion (that is, a natriuretic hormone[22]). Increased circulating amounts of this substance may induce hypertension as a side effect[1]. In addition to their possible significance for pathophysiology, our observations imply that, even in normal circumstances, the activity of Na^+ pumps may be directly modulated *in vivo* by humoral substances.

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Nonspecific inhibitor released by T acceptor cells reduces the production of interleukin-2

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Mice exposed to contact sensitizing agents such as oxazolone (ox) or picryl (pic) derivatives develop specific immunological suppression to these substances which results from the activation of a circuit of T suppressor cells[1–3]. However, one of the final immunological events in the T suppressor cell circuit is the release of a nonspecific, H–2 unrestricted inhibitor by I–J$^+$, Lyt-2$^+$ cyclophosphamide-sensitive T acceptor cells (Tacc)[3–7]. This nonspecific inhibitor (nsINH) can block the passive transfer of contact sensitivity and depress DNA synthesis by lymphocytes exposed to mitogens and alloantigens but the molecular mechanism is poorly understood. We provide evidence here that the production of interleukin-2 (IL-2)[8–15] by stimulated lymphoid cells is suppressed when the cells have been exposed to nsINH.

The T suppressor cell circuit, which has an important role in the modulation of immune responses to ox and pic derivatives, involves Lyt-1$^+$2$^+$ hapten-specific T suppressor cells producing hapten-specific T suppressor factor (TsF), which arms the Tacc; the armed Tacc population then liberates nsINH when exposed to hapten-modified (oxazolonated or picrylated) lymphoid cells corresponding in hapten specificity to TsF and bearing the same I–J subregion as the source of TsF[3–7]. Our initial observation was that nsINH diminishes proliferative responses to con-

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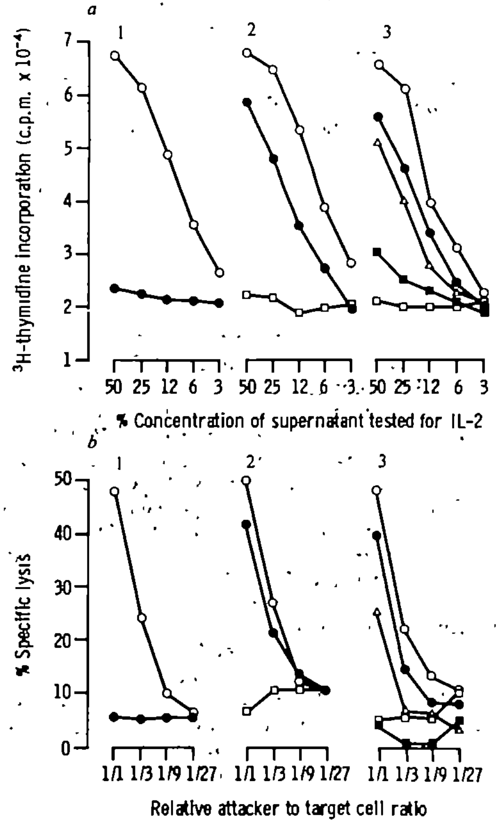

Fig. 1 Reduced IL-2 activity in supernatants of Con A-activated mouse spleen cells exposed to nsINH. A lymphoid cell suspension was prepared by pressing BALB/c spleens through a fine mesh screen into RPMI 1640 medium. Erythrocytes were lysed using Boyle's solution[27]. The lymphoid cells were mixed with various concentrations of the nsINH supernatant or with 100% of the control supernatants (10^7 cells ml^{-1}); Con A (5 μg ml^{-1}) was added and the suspension was distributed in 4-ml aliquots into 12-well flat bottom Linbro tissue culture plates (Flow). Similarly, 4-ml aliquots of the same cells (10^7 cells ml^{-1}) were resuspended in RPMI 1640 medium with (positive assay control) or without (negative assay control) Con A (5 μg ml^{-1}), and distributed into the plates. After 4 h incubation, the supernatant was carefully removed and discarded. Fresh serum-free RPMI 1640 medium (without Con A) was added and the supernatant was collected after a further incubation for 20 h and tested for IL-2 activity in two different assays. The presence of IL-2 was measured initially using 4 day Con A blast cells essentially following ref. 14 (Fig. 1*a*). BALB/c Con A blast cells (10^6 cells ml^{-1}) were cultured in 0.2 ml supplemented RPMI 1640 medium in the presence of various concentrations of the supernatants to be tested for IL-2 activity. Cultures were set up in quadruplicate in 96-well Titertek tissue culture plates. Five hours before collection, each well was pulsed with 1 μCi [*Me*-^{3}H]thymidine. The cultures were collected after 25 h and tritiated thymidine incorporation was determined. In *a*, the supernatants tested for the IL-2 activity were: panel 1: supernatant from untreated spleen cells, the negative assay control (●), supernatant from Con A-activated spleen cells, the positive assay control (○); panel 2: supernatants from Con A-activated spleen cells exposed to a 100% nsINH supernatant (□) or to 100% control supernatants prepared with the omission of either antigen (○) or TsF (●) (see Table 1); panel 3: supernatants from Con A-activated spleen cells exposed to different concentrations of the nsINH supernatant—50% (□), 25% (■), 12% (△), 6% (●), 3% (○). The abscissa indicates the concentration of the supernatants tested for IL-2. The ordinate indicates the IL-2 activity as measured by the tritiated thymidine incorporation by Con A blasts. Con A blasts incubated in culture medium only for 25 h incorporated tritiated thymidine to a similar extent as the negative assay control (19,555 ± 2,721 c.p.m.). In *b*, the same supernatants were assayed for IL-2 activity by their capacity to sustain the growth and function of cultured CTL[9,16]. The following modification of the original method described in ref. 16 was used. Female CBA (H-2^k) spleen cells from untreated mice (4×10^6 cells ml^{-1}) were co-cultured with irradiated (2,000 R, ^{60}Co source) NZB (H-2^d) stimulator lymphoid cells (2×10^6 cells ml^{-1}) in supplemented RPMI 1640 medium with 5×10^{-5} 2-mercaptoethanol. Cultures were seeded in 12-well flat bottom Linbro tissue culture plates in a total volume of 4 ml per well. After 5 days, dead cells were removed by centrifugation over Ficoll/Isopaque[28]. Live cells (0.25×10^6 cells per ml) were co-cultured with fresh irradiated NZB stimulator lymphoid cells (10^6 cells per ml) for another 2 days. The cells were then collected, purified over Isopaque/Ficoll[28] and plated in 24-well flat bottom Linbro tissue culture plates (Flow). Cells (2×10^4 cells ml^{-1}) were incubated in various concentrations of supernatants to be tested for the IL-2 activity or in culture medium for 3 days. For the cytotoxicity assay, the cells were washed once and resuspended in 0.5 ml supplemented RPMI 1640 medium; 0.1-ml triplicates of four threefold dilutions were set up in 96-well U-bottom Microtiter plates (Sterilin) and incubated in a total volume of 0.2 ml with P815 (H-2^d) target cells (3×10^3 cells per well) labelled with Na$_2$[^{51}Cr]O$_4$ (specific activity: 100–400 mCi per mg Cr, Amersham, UK). The Cr-release was stopped by centrifugation after 3 h incubation by centrifugation (300*g*, 10 min, 4 °C). The Cr-release c.p.m. were determined by counting a 100-μl supernatant from each well in a gamma counter.

$$\%\ \text{Specific lysis} = \frac{\text{experimental c.p.m.} - \text{medium control c.p.m.}}{\text{maximum release c.p.m.} - \text{medium control c.p.m.}} \times 100$$

Medium control c.p.m. were obtained from 100-μl supernatant aliquots taken from wells containing radiolabelled target cells only. Maximum release c.p.m. were obtained from wells containing radiolabelled target cells in 5% Triton X-100 (BDH). *b* Shows the cytotoxic potential of murine secondary CTL incubated for 3 days in the presence of a 25% concentration of the supernatants tested for the IL-2 activity and the panels are as in *a*. Relative attacker to target cell ratios are indicated on the abscissa and % specific lysis on the ordinate. Very similar results were obtained with other concentrations of the supernatants and the cytotoxic cells were shown to be T lymphocytes with monoclonal anti-Thy-1.2 antibody (Olac) and complement (unpublished data).

canavalin A (Con A) only when added less than 8 h after the proliferative stimulus[3,7]. However, the effect of nsINH could be overcome by adding exogenous IL-2 but not by increasing the concentration of Con A. These findings prompted investigation of the possibility that the effect of nsINH could be due to the relative lack of IL-2 in cultures of stimulated lymphoid cells which had been treated with nsINH. In the first set of experiments we prepared control supernatants and supernatants containing nsINH and tested them in the DNA synthesis assay (Table 1). The nsINH supernatant (50% concentration) of BALB/c origin depressed Con A-induced DNA synthesis to ~40% of the control value in syngeneic BALB/c (Table 1) or in allogeneic B10.A or CBA (data not shown) indicator cells, while control supernatants were inactive.

The second set of experiments examined the ability of nsINH supernatants to influence Con A-induced IL-2 production by lymphoid cells. BALB/c spleen cells were suspended in culture medium or in 100% control supernatants or in different concentrations of a supernatant with nsINH activity and exposed to Con A. After a 4-h incubation, the culture fluids were removed and replaced with serum-free RPMI 1640 medium. The production of IL-2 by the cells was assessed by measuring the IL-2 activity in the culture medium after a further 20-h incubation using both proliferation[14] and functional[9,16] assays for IL-2 (Fig. 1). The results, confirmed in seven independent experiments, showed that the production of IL-2 by stimulated lymphoid cells was at least 15-fold lower as estimated by the biological assays for IL-2, when the cells were exposed to 25–100% concentration of nsINH supernatant. Moreover, the action of nsINH was nonspecific and H-2-unrestricted and was not limited to Con A-induced IL-2 production. Table 2 shows that nsINH of BALB/c or CBA origin inhibited the production of IL-2 in supernatants from Con A- or alloantigen-stimulated BALB/c or CBA spleen cells.

Table 1 Nonspecific inhibitor depresses the DNA synthetic response to Con A in spleen cells

Preparation of supernatant Tacc (4-day ox spleen/lymph-node cells)	TsF (anti-picryl)	Antigen (picrylated spleen cells)	Additions to test culture	^{3}H-thymidine incorporation (c.p.m. ± s.d.)	% of Con A response
			—	57±10	
			Con A (2 μg ml^{-1})	45,800±379	100 (standard)
+	+	−	Con A+50% control supernatant (no antigen)	44,857±2,017	98
+	−	+	Con A+50% control supernatant (no TsF)	41,693±4,970	91
+	+	+	Con A+50% nsINH supernatant	17,489±341	38

The nsINH is released by Tacc armed with TsF and triggered by antigen[3–7]. Six to nine-week-old female BALB/c mice were used throughout. The anti-picryl T suppressor factor (pic TsF) was produced by 48-h cultures of lymphoid cells from mice injected and then painted with picryl derivatives[6,7,24]. The lymphoid cells (10^7 cells per ml) were incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO$_2$ in supplemented RPMI 1640 medium (Flow). The supplemented RPMI 1640 medium contained 5% fetal calf serum (Flow), 2×10^{-3} M L-glutamine (Sigma), 1×10^{-3} M HEPES (Flow), 100 IU ml^{-1} penicillin and 100 μg ml^{-1} streptomycin (Glaxo). Nylon wool purified T cells[25] from spleen and lymph nodes taken 4 days after painting mice with oxazolone were used as the Tacc population[6,7]. The spleen cells were picrylated by the method of Shearer[26]. Control supernatants and supernatants containing nsINH were prepared following refs 6 and 7. In brief, the Tacc population was incubated in a supernatant containing pic TsF (25×10^6 cells per ml) for 1 h and shaken occasionally. The armed Tacc were recovered by centrifugation and mixed with a third of their number of picrylated cells, centrifuged, resuspended in supplemented RPMI 1640 medium (25×10^6 Tacc ml^{-1}) and incubated for 2.5 h. The suspension was centrifuged and the supernatant filtered through a 0.22-μm Millex-GS filter (Millipore). Control supernatants were prepared by omitting either picrylated cells or pic TsF. These supernatants were tested in the DNA synthesis assay[7]. Briefly, spleen cells from untreated mice (7.5×10^5 cells ml^{-1}) were cultured in 0.2 ml of supplemented RPMI 1640 medium with 2×10^{-5} M 2-mercaptoethanol (type I, Sigma) and 2 μg ml^{-1} Con A (Pharmacia). (The batch of fetal calf serum was selected to be nonmitogenic for mouse cells so that DNA synthesis in cultures untreated with mitogens was less than 2% of DNA synthesis in mitogen-treated cultures.) Cultures were set up in quadruplicate in 96-well Titertek tissue culture plates (Flow). The final concentration of supernatants in the assay was 50%. Five hours before collection, each well was pulsed with 0.5 μCi [*Me*-^{3}H]thymidine (specific activity: 5 Ci mM^{-1}, Cat. no. TRA.120, Amersham). The plates were collected after 72 h using a multicell cell collector (Scatron) and Titertek collector filters (Flow). Tritiated thymidine incorporation was determined by liquid scintillation counting in 2 ml Micellar Scintillator (Nuclear Enterprises).

It was possible that nsINH might act indirectly by inducing the release of an IL-2 inhibitor[17], which could block the action of IL-2. To exclude this possibility, the IL-2 supernatant (the positive assay control in Fig. 1) and the supernatant from Con A-activated spleen cells exposed to nsINH were subject to preparative isoelectric focusing (Fig. 2). In the control supernatant, the IL-2 activity was recovered as a single peak in the *p*H range of 3.8 to 4.6; peak activity migrated with an isoelectric point of 4.1 in keeping with ref. 14. The results of Watson *et al.*[18], who reported two distinct peaks (*p*I 4.3 and 4.9) of murine IL-2 activity, may reflect the known heterogeneity of IL-2 which is due in part to variable glycosylation. However, no IL-2 activity was recovered from the supernatant from Con A-activated cells exposed to nsINH. Moreover, the data in Table 3 show that inactive supernatant did not affect the ability of IL-2 to promote cell proliferation. It was concluded that nsINH reduced the production of IL-2 by lymphoid cells and did not induce an inhibitor of the action of IL-2. The nsINH was not nonspecifically toxic to lymphoid cells because the cell viability assessed by trypan blue dye exclusion of normal unstimulated spleen cells was unaffected by the presence of nsINH. Moreover, the growth of lymphoid cell lines BW 5147 (ref. 19), EL4 (ref. 20) and P3-NSI/1-Ag4-1 (ref. 21) was not influenced by nsINH. Finally, nsINH had no effect on Con A-induced cell proliferation when added after the first 8 h of culture[3,7] and its effect when added at the beginning of culture was abolished by exogenous IL-2. For instance, in an experiment in which spleen cells, stimulated by Con A, incorporated 94,963±8,174 c.p.m. of tritiated thymidine on day 3, but only 25,771±3,325 c.p.m. in the presence of 50% nsINH, cell proliferation was completely restored (96,008±7,027 c.p.m.) by adding 20% supernatant containing exogenous IL-2.

Preliminary data suggested that the nsINH, which had an isoelectric point of 6.4–6.9 and a molecular weight of 40–60,000, did not influence the differentiation of primary or secondary cytotoxic T lymphocytes (CTL)[3,7]. These data are compatible with the view that a distinct soluble factor but little or no IL-2 are required to induce differentiation of effector CTL from resting precursors[22,23]. The present T suppressor cell circuit[3–7] involving the liberation of nonspecific inhibitor of the

Table 2 The action of nonspecific inhibitor is not H-2-restricted

	IL-2 assay (c.p.m. ± s.d.)		
		Nonspecific inhibitor present (50% v/v) Origin of the nonspecific inhibitor	
Origin of IL-2	Nonspecific inhibitor absent	BALB/c	CBA
Alloantigen-stimulated BALB/c	27,211±3,185	5,824±795	5,762±823
Con A-stimulated BALB/c	45,895±3,455	5,956±1,491	6,167±1,002
Alloantigen-stimulated CBA	29,286±1,920	5,837±921	5,626±813
Con A-stimulated CBA	49,186±3,696	6,170±504	5,844±879

Mixed lymphocyte cultures were set up as described elsewhere[7]. In brief, BALB/c or CBA spleen cells at a final concentration of 5×10^6 cells ml^{-1} were co-cultured with equal numbers of irradiated (2,000 R, ^{60}Co source) allogeneic stimulator CBA or BALB/c spleen cells in the absence or presence of nsINH. After a 3-day incubation, the supernatant was removed and replaced with fresh culture medium, which was collected after a further 1 day incubation and tested for IL-2 activity. The preparation of supernatants from Con A-stimulated spleen cell cultures and the testing of the IL-2 activity of the supernatants by their capacity to promote the proliferation of 4-day Con A blasts are described in the legend to Fig. 1. The data in Table 2 show IL-2 production as measured by the proliferation of 4-day Con A blasts incubated for 25 h in the presence of a 25% concentration of supernatant. Similar results were obtained with other concentrations (50, 12, 6%) of the supernatants. The incorporation of tritiated thymidine into Con A blasts incubated for 25 h in culture medium only was 5,407±807 c.p.m. Alloantigen- or Con A-stimulated cells liberated approximately the same amounts of IL-2 as the same cells exposed to control supernatants prepared with the omission of either antigen of TsF (data not shown).

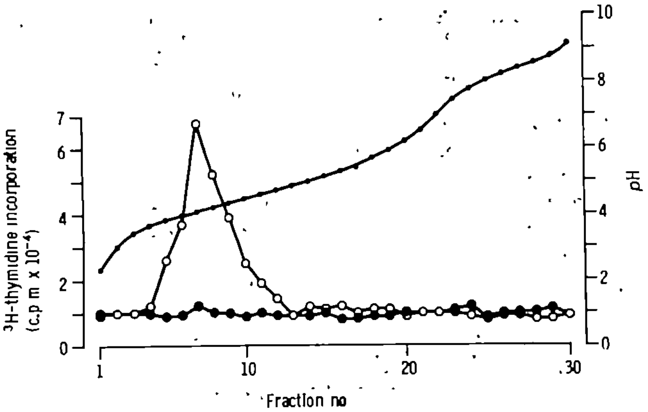

Fig. 2 IL-2 cannot be isolated by preparative isoelectric focusing from supernatants of Con A-activated spleen cell exposed to nsINH. Preparative isoelectric focusing was carried out in a flat-bed of Ultradex gel (LKB) using the LKB 2117 Multiphor Apparatus following refs 14 and 18. Briefly, a final concentration of 4% Pharmalyte ampholites (pH 3–10, Pharmacia) was added to samples, which were electrophoresed for 16 h at a constant power of 8 W. The gel was then sectioned into 30 slices, each slice was eluted with 2 ml H_2O and the pH was determined. Each sample was dialysed against phosphate-buffered saline, finally against RPMI 1640 medium and assayed for biological activity using Con A blast cells as described in Fig. 1 legend. The supernatants were from Con A-activated spleen cells (○) and from Con A-activated spleen cells exposed to a 100% supernatant containing nsINH (●). The pH curve is given for the supernatant from Con A-activated spleen cells. Fractions 4–15 of the second supernatant had the same pH and the difference between the pH of corresponding samples of remaining fractions was less than 0.2.

Table 3 Absence of an inhibitor of the action of IL-2 in a supernatant from Con A-activated spleen cells exposed to nsINH

Additions to test culture	^{3}H-thymidine incorporation (c.p.m. ± s.d.) Medium	Supernatant
—	7,624±230	7,508±1,933
5% IL-2	21,001±1,600	20,589±763
10% IL-2	42,481±1,885	42,230±1,089
20% IL-2	57,381±2,779	55,692±1,207
30% IL-2	64,607±4,577	63,792±7,345
40% IL-2	79,773±2,834	77,865±774

Various final concentrations of an IL-2 supernatant (the positive control in the experiment described in Fig. 1) were prepared by diluting in either RPMI 1640 medium or in a supernatant, from Con A-activated spleen cells exposed to a 100% supernatant containing nsINH, in which IL-2 activity could not be detected directly (Fig. 1) or after preparative isoelectric focusing (Fig. 2). The dilutions were assayed for IL-2 activity by their capacity to promote the proliferation of 4-day Con A blasts using the technique described in the legend to Fig. 1.

production of IL-2 could provide a mechanism which limits the entry of new cells proliferating under the action of IL-2 into an immune response.

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Influenza A specific cytotoxic T-cell clones that do not recognize viral glycoproteins

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Since the demonstration that murine cytotoxic T cells generated during a secondary immune response to influenza A viruses were composed of cross-reactive and subtype-specific populations[1–6], it has become of interest to define the viral components responsible for each pattern of recognition. One approach is by the isolation of individual clones of cytotoxic T cells[6,7] which can then be tested for recognition of natural or laboratory-induced virus variants of known genetic composition. We describe here a series of influenza A virus subtype-specific clones raised against the recombinant X31(H3N2)[8] virus, which contains the haemagglutinin (HA) and neuraminidase (NA) from the 1968 virus, and all its remaining genes from the 1934 virus[9]. We show here that the viral gene responsible for the determinant recognized by these cytotoxic T-cell clones codes for neither the haemagglutinin nor neuraminidase glycoproteins, that it segregates independently of HA and NA during the formation of recombinant viruses, and that it undergoes independent genetic change.

The virus X31 (H3N2) is a recombinant between A/Aichi/1968 (H3N2) and A/PR/8/1934 (H1N1). Clones able to recognize the recombinant virus X31 (H3N2) but not A/USSR/90/77 (H1N1)-infected target cells are here called subtype-specific. The subtype-specific cytotoxic clones examined here all show either no or very little activity on A/USSR compared with X31, and were selected on this basis for closer inspection. In each experiment a cross-reactive clone, T6-11-5-1, or polyclonal line D, able to kill targets infected with any influenza A virus, served as a positive control, as different viruses tend to infect target cells with varying efficiency.

Figure 1 shows the spectrum of activity of clone A3.1. It reveals that target cells infected with A/PR/8/1934 are recognized but that A/Aichi/1968 is not, while the cross-reactive control kills both equally well. As A/PR/8/1934 contributes all the genes to X31 other than the HA and NA genes[9], the subtype-specific component being recognized seems not to be the haemagglutinin or neuraminidase surface glycoproteins. Figure 1 also shows that the virus component recognized underwent genetic change between 1943 and 1946 because the 1943 virus infected cells are killed as efficiently by A3.1 as by the cross-reactive control, whereas the 1946 and 1947 virus infected targets are not killed at all by A3.1 but are killed very efficiently by the cross-reactive control. The influenza B/Hong Kong/8/73 infected target acts as a specificity control for both cross-reactive and subtype-specific populations. Four other clones (three from a separate culture), derived in the same way as A3.1, showed the same pattern (Table 1), killing A/PR/8/1934 targets but not A/Eng/42/1972 targets (which is antigenically similar to A/Aichi/1968).

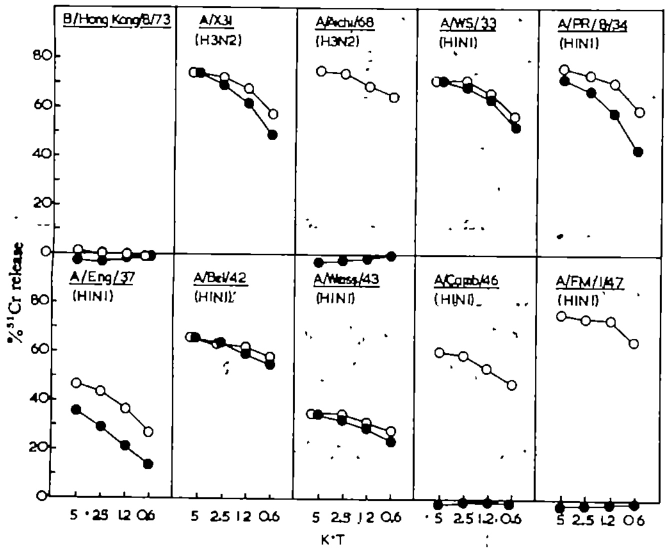

Fig. 1 Spectrum of activity of clone A3.1 on target cells infected with various influenza A viruses (●—●); line D effectors (○—○) act as cross-reactive control. Combined results from two experiments. T-cell clones: the derivation and maintenance of clone A3.1 and clones depicted in the other figures will be described in detail elsewhere (A.R.M.T. and P. M. Taylor, manuscript in preparation). Briefly, polyclonal cytotoxic lines were established by repeated stimulation *in vitro* of spleen cells from intranasally primed donor mice with X31 infected, irradiated, syngeneic spleen cells. After two stimulations growth factors were added each time as 20% v/v supernatant from concanavalin A pulsed rat spleen cells (CAPS/N). After at least two more stimulations *in vitro*, the cells were cloned by limiting dilution at least once at 0.5 cells per well onto an X31 infected syngeneic spleen cell feeder layer. Growing clones were transferred to 2-ml Costar wells after 7–14 days and maintained in Costars by stimulation every 3–5 days in the presence of 20% CAPS/N. The subtype-specific clones originated from X31 primed mice and were selected and maintained using X31 *in vitro*. Clones A3.1 and A3.6-5.4 were derived from the same cell line, B10.7, B8.3 and C2.2 from a different line. The cross-reactive control clone T6–11-5-1 and the polyclonal line D were derived from A/JAP/305/57 (H2N2) primed mice and were selected by X31 *in vitro*. All cells were grown in RPMI/1640 medium supplemented with 10% fetal calf serum, penicillin, streptomycin, glutamine and 5×10^{-5}M 2-mercaptoethanol. All the subtype-specific clones described show *H–2* restricted recognition, requiring the class I molecule D^b on the target cell surface[24]. The cytotoxicity assay was performed as described previously[24]. Cloned cytotoxic T cells were incubated, at the ratios indicated, with ^{51}Cr-labelled target cells infected with the test virus (50–400 μl infectious allantoic fluid per 10^7 cells per 90 min) in a 6-h assay. The per cent specific ^{51}Cr released from lysed target cells was calculated as follows:

$$\frac{\text{(Release in presence of cytotoxic T cells} - \text{medium release)}}{\text{(Triton release} - \text{medium release)}}\times 100$$

All points measured in duplicate where four different killer : target ratios (K : T) were tested or quadruplicate where 1K : T was tested. Target cells were EL4 cells maintained as an ascitic tumour *in vivo*. Spontaneous ^{51}Cr release in presence of medium (SR) = 13–34%.

One clone, A3.6-5.4, from the same culture as A3.1, was tested on a number of viruses of the H3N2 subtype isolated between 1968 and 1979 (Fig. 2). It was unable to kill targets infected with any of them, whereas the cross-reactive clone T6-11-5-1 was positive on all the A virus infected targets.

To be sure that the determinant from the 1934 virus being recognized was encoded by a gene other than HA or NA, we tested clones on targets infected with a variety of recombinant viruses produced by crossing post-1968 H3N2 viruses with either A/PR/8/1934 (H1N1) or A/Bel/1942 (H1N1) (Fig. 3). Of these X31, MRC11 and X47 are antigenically H3N2, MRC1 and Mem/Bel 102 are H3N1. As illustrated by clone A3-6-5-4 (Fig. 3) the X31 and MRC1 infected targets are killed, but not the MRC11, Memphis/Bel 102, or X47 targets, whereas the cross-reactive control kills all the targets infected with recombinant A viruses.

We have found that polyclonal cytotoxic T-cell lines originating from C57B/6 mice primed with X31 and maintained *in vitro* by stimulation with X31, initially show characteristic cross-reaction on all influenza A virus-infected targets[1], but after several stimulations give rise to subtype-specific clones with high frequency (data not shown). There is thus, in the C57B/6 strain at least, a selection *in vitro* for the A virus subtype specific subset using this protocol. The clones discussed here are from such an *in vitro* selected population.

Previously, attention has naturally focused on the haemagglutinin molecule as a source of virus subtype-specific determinants recognized by B (refs 10, 11) and T cells[12], the more so because it has been shown by several workers that secondary cytotoxic T-cell responses induced *in vitro* with purified viral haemagglutinin show subtype-specificity, and the determinants responsible map to the HA gene when tested on appropriate recombinant viruses[2,13]. Haemagglutinin-specific cytotoxic T cells must therefore exist.

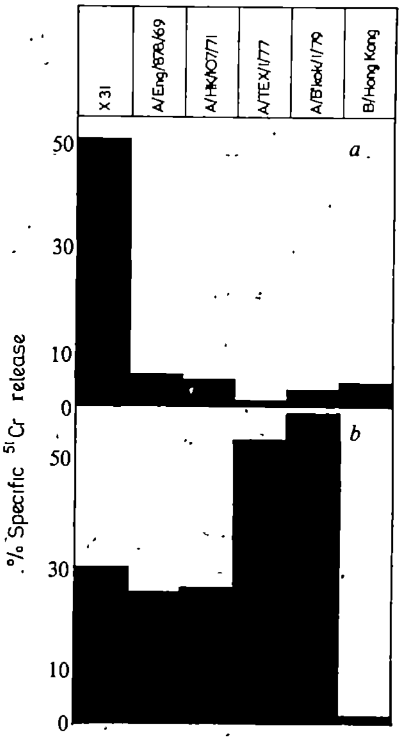

Fig. 2 *a*, Activity of clone A3.6-5.4 on target cells infected with post-1968 H3N2 influenza A viruses. *b*, Cross-reactive control clone T6-11-5-1. Combined results from two experiments. Thioglycollate induced PEC as targets. SR = 25–42%.

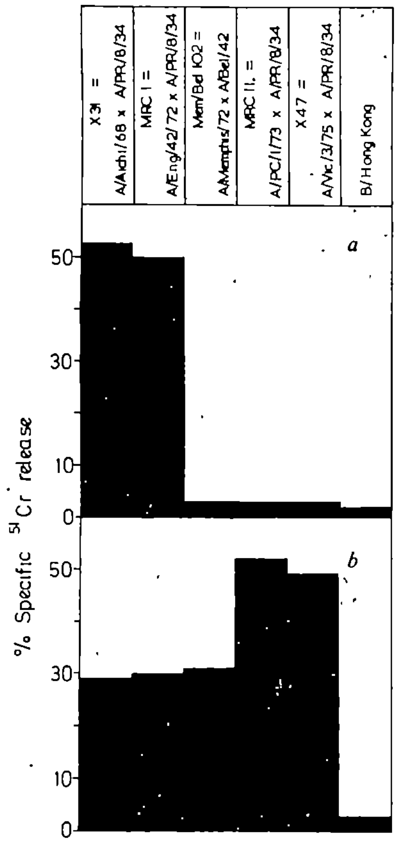

Fig. 3 *a*, Activity of clone A3.6-5.4 on recombinant viruses. *b*, Cross-reactive control clone T6-11-5-1. Combined results from two experiments. Thioglycollate induced PEC as targets. SR = 19–52%.

It was thus a surprise to find that all the T-cell clones isolated by us in the way described (see Fig. 1 legend) do not recognize either of the viral surface glycoproteins. The evidence for this is twofold. First, of the two viruses which contribute genes to X31, only the A/PR/8/1934 is recognized despite the fact that X31 does not receive its HA and NA genes from A/PR/8/1934. Second, when tested on a variety of recombinant viruses of known surface glycoprotein constitution, X31 (H3N2) and MRC1 (H3N1) are killed, but Mem/Bel 102 (H3N1), MRC11 (H3N2) and X47 (H3N2) are not (Fig. 3), showing that the viral gene responsible for recognition of infected target cells by clone A3.6-5.4 can segregate independently of the HA and NA genes during the formation of these five different recombinant viruses.

Braciale *et al.*[7] describe a series of murine cytotoxic T-cell clones derived using a related protocol with A/JAP/305/1957 (H2N2) virus. A similarly high frequency of clones showed subtype specificity, and these could be dvided into those able to recognize targets infected only with 1957 H2N2 virus and those able to recognize both the 1957 and 1967 H2N2 viruses. Representatives of both groups when tested on recombinant viruses of known HA and NA composition seemed to be HA specific. However, it is possible that a non-HA gene responsible for recognition could have segregated by chance with the HA gene in the two recombinant viruses tested with a probability of 1/4.

Table 1 Activity of T-cell clones on target cells infected with different influenza A viruses

Clone	Virus K:T	% Specific ^{51}Cr release			
		X31	A/Eng/42/72	A/PR/8/34	B/Hong Kong
A.31	2..5	46	4	30	1
A3.6-5.4	2.5	53	2	30	2
B8.3	2.5	46	3	26	2
B10.7	2.5	45	2	27	1
C2.2	2.5	37	3	16	1
T6-11-5-1 Control	3	29	30	16	2

Thioglycollate-induced peritoneal exudate cells (PEC) used as targets. SR = 16–43%.

Lamb *et al.*[14] have described six human H3N2 influenza-subtype specific T-cell clones. All six gave a specific proliferative response only with either purified viral HA or NA, whereas five cross-reactive clones gave specific responses only with purified matrix protein (M) or nucleoprotein (NP). This result may, like our own, reflect the technique of *in vitro* selection used, and does not rule out subtype-specific determinants on viral components other than HA and NA.

The determinant we have defined with clone A3.1 undergoes genetic change, as shown in Fig. 1, between the 1943 and 1946 virus isolates. It is of interest that the timing of this change coincides with a major change in the antigenicity of the H1N1 nucleoprotein as shown by van Wyke *et al.*[15] using monoclonal antibodies and Schild *et al.*[16] using rabbit antisera to purified NP. Furthermore Schild *et al.*[17] identified the origins of the NP in X31, X47 and MRC11, three of the recombinants we used. X31 is the only virus to receive the A/PR/8/1934 NP gene, and the only one of these three recognized by clone A3.6-5.4. However, from the results presented here none of the virus proteins other than the HA and NA glycoproteins can be excluded as the target for these clones. Also, although NP has been shown to be expressed on the surface of infected cells[18,19], there are also reports that NS1[20] and M protein[21] can be detected on the surface of infected cells, albeit for the latter in very low quantities when compared with HA[22].

A recent report[23] implicates the polymerase 3 gene as an absolute requirement for recognition by an isolated subtype-specific cytotoxic T-cell clone. This clone was derived by stimulation with A/PR/8/1934, and the recombinant A virus recognition data conclusively showed that neither the HA nor NA genes from A/PR/8/1934 were required. Thus far it resembles clone A3.1; however, the former recognized only targets infected with 1934 and 1935 H1N1 viruses, while clone A3.1 recognizes all H1N1 viruses isolated between 1933 and 1943. The determinants detected by these two A virus subtype-specific clones may therefore be different.

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Haemagglutinin of influenza virus expressed from a cloned gene promotes membrane fusion

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Enveloped animal viruses enter and infect cells by a process involving fusion of the viral membrane with a cellular membrane. In some cases (for example, Sendai virus[1]), the fusion event occurs at the plasma membrane; for many other viruses including influenza, entry occurs through the membranes of intracellular vesicles such as endosomes or lysosomes, where the fusion is triggered by the endogenous low *p*H[2–6]. This *p*H-dependent fusion activity has been studied *in vitro* using as targets cultured cells[7–9], erythrocytes[9,10] and liposomes[11–13]. Fusion is a function of the viral surface glycoproteins[12] and occurs at a threshold *p*H that is characteristic of each virus species and strain[8]. In the case of influenza virus, there is strong evidence that the haemagglutinin glycoprotein has a key role in both virus infectivity and fusion activity[9,12,14–16]. Both processes require a post-translational proteolytic cleavage of the haemagglutinin precursor, HA0, into the active form of the molecule, HA, which consists of two disulphide-bonded subunits, HA1 and HA2[17,18]. A new N-terminus is generated on the HA2 subunit, the C-terminus of which is embedded in the virus membrane[19]. At the low *p*H values required for fusion the cleaved HA undergoes a conformational change exposing this previously buried hydrophobic N-terminus of HA2[20], possibly enabling it to interact with the target membrane. While it has been established that HA is necessary for fusion, it is unclear whether HA alone is sufficient or whether other viral proteins are involved[21]. Here we use cells expressing HA from a cloned copy of the HA gene inserted into a recombinant simian virus 40 (SV40) vector[22] to demonstrate that the HA molecule displays fusion in the absence of any other influenza virus-encoded components. These results open the possibility of using HA-mediated membrane fusion as a system to deliver foreign molecules into mammalian cells.

Simian CV-1 cells infected with a recombinant SV40 virus (SVEHA3) which contains the HA gene from the Japan strain of influenza virus (A/Japan/305/57) express at the cell surface large quantities of a HA glycoprotein that is indistinguishable in its antigenic, physical and biological properties from authentic influenza viral HA0[22]. Figure 1 shows that it is possible to cleave the HA0 into HA1 and HA2 subunits by briefly treating the cells with a low concentration of trypsin.

Table 1 Comparison of the *p*H dependence of fusion of CV-1 cells by SV40-HA recombinant viruses and influenza virus

Virus	Trypsin	*p*H 6.0	5.5	5.3	5.1	4.9
SVEHA3	−	−	−	−	−	−
	+	−	−	+/−	+	+
SVEHA20-A$^-$ (anchor minus)	−	−	−	−	−	−
	+	−	−	−	−	−
SV40	−	−	−	−	−	−
	+	−	−	−	−	−
Mock	−	−	−	−	−	−
	+	−	−	−	−	−
Influenza (Japan)	−	−	−	−	−	−
	+	−	−	+/−	+	+
FFWO with influenza (Japan)	−	−	−	+/−	+	+

CV-1 cells were infected with the various viruses as described previously[22]. 24 h later the *p*H dependence of fusion of the cells was assayed, with or without prior treatment with trypsin, by the protocol described in Fig. 2 legend using FM adjusted to the indicated *p*H. The assay for fusion-from-without (FFWO) was performed as described previously[8] using both CV-1 and BHK cells as targets. − Indicates that no fused cells were observed, + indicates that all of the cell nuclei were present in polykaryons and +/− indicates that some but not all nuclei were present in polykaryons.

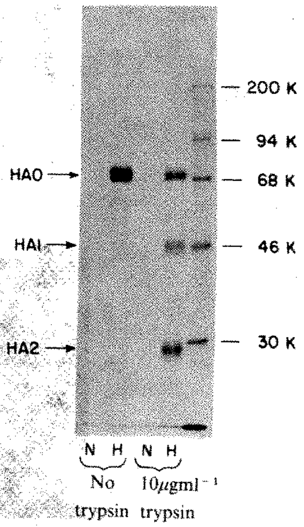

Fig. 1 Proteolytic cleavage of HA0 on the surface of SVEHA3-infected CV-1 cells. CV-1 cells were infected with SVEHA3 as described previously[22]. 48 h later the cells were labelled for 1 h with ^{35}S-methionine and then washed twice with serum-free medium. Medium containing 10 μg ml^{-1} trypsin (or lacking trypsin as control) was added to the monolayers which were then incubated for 15 min at 37 °C. Cell extracts were prepared, immunoprecipitated and analysed by SDS-polyacrylamide gel electrophoresis as previously described[22]. Autoradiography was for 12 h. N, normal rabbit serum; H, rabbit anti-HA serum. Molecular weight markers are indicated on the right.

Monolayers of CV-1 cells were infected with SVEHA3 to obtain expression of HA0 at their surface. When treated with trypsin and briefly exposed to *p*H 5.0, the cells fused to form giant polykaryons (Fig. 2*a*). This fusion was observed from 24 h post-infection, when the cells contained ~10^7 HA0 molecules, until just before cell lysis, which occurred at about 72 h post-infection when the cells contained ~10^9 molecules of HA0[22]. No fusion occurred unless either the infected cells were exposed to low *p*H (Fig. 2*b*, *d*) or the trypsin treatment was omitted (Fig. 2*c*, *d*). CV-1 cells that had been mock-infected or infected with wild-type SV40 did not fuse (Table 1). Thus, the HA expressed from the SV40-HA vector if activated by cleavage into HA1 and HA2 subunits, is capable of inducing low *p*H-dependent membrane fusion in the absence of other influenza viral components.

The *p*H dependence of the cell–cell fusion in SVEHA3-infected cells is virtually identical to that observed for fusion of influenza-infected CV-1 cells or for fusion-from-without of CV-1 or BHK cells by intact Japan virus (Table 1). Thus, the membrane environment in which the HA is anchored (the plasma membrane of cultured cells or the viral membrane) has little or no effect on the *p*H threshold for fusion. This is consistent with the observation of Skehel *et al.*[20] that the conformational change in isolated HA occurs at the same *p*H as does activation of fusion by the intact influenza virus[8].

Having established that the HA is necessary and sufficient for *p*H-dependent fusion, tests were performed to determine whether the protein had to be anchored to a membrane. The HA can be obtained, free of lipid and detergent, in three water-soluble forms: (1) as rosettes or protein micelles, in which the HA molecules are joined through their hydrophobic tails[23], (2) as free glycoprotein molecules, released from the virus membrane by digestion with bromelain (BHA)[24], and (3) as glycoprotein secreted from CV-1 cells infected with a recombinant vector (SVEHA20-A$^-$) containing a modified HA gene that lacks the DNA sequences coding for the C-terminal hydrophobic anchor (see accompanying paper[25]). The addition of HA rosettes or BHA spikes to monolayers of BHK cells did not cause any *p*H-dependent cell–cell fusion despite the fact that these cells could be completely fused from without by intact virions containing 50-fold less HA protein[8]. Furthermore, no fusion occurred when CV-1 cells infected with the anchor-minus mutant were treated with trypsin and exposed to low *p*H (Table 1). These results indicate that HA must be attached to a lipid matrix such as the viral envelope or the plasma membrane for fusion to take place.

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It has long been known that HA is responsible for binding influenza virions to cell-surface receptors[26]. More recently, it has become apparent that HA has a second major role in virus entry—that of fusion of the virus membrane with the membrane of endocytic vacuoles or lysosomes[2]. We have demonstrated that a HA produced from a cloned DNA copy of the RNA gene not only expresses the binding function[22], but also displays the *p*H-dependent fusion activity. These results show that no other influenza virus gene product is required for either of these functions. The binding and fusion activities, although carried out by the same protein, occur independently of each other. Fusion does not require sialic acid receptors in the target membrane[11,13] and binding to such receptors does not require the cleaved form of the HA that is obligatory for fusion[22]. Examination of the three-dimensional structure of the HA molecule[27] indicates that the two functions are separated into different physical domains of the protein. The proposed receptor binding sites are located at the tips of the spikes while the hydrophobic N-terminus of HA2, which becomes exposed at low *p*H (ref. 20 and J. J. Skehel, unpublished) and which has been implicated as the 'fusion peptide'[27–30], is found in the stem region at the base of the molecule close to where HA is anchored to the membrane.

The requirements for HA-mediated membrane fusion are (1) that the molecule be cleaved into its active form, (2) that it be anchored via its C-terminus into a lipid bilayer and (3) that it be exposed to low *p*H. A plausible mechanism satisfying these requirements and incorporating previous hypotheses[27–30] involves the two hydrophobic regions of the protein: one of these at the C-terminus anchors the protein into the membrane of the virus (or the infected cell); the second hydrophobic peptide at the N-terminus of HA2 is exposed by the conformational change at low *p*H and inserts into the target membrane. This conformational change cannot occur unless the protein has been cleaved. The HA protein now forms a bridge between the two membranes, bringing the lipid bilayers into close apposition and thus facilitating their fusion. Confirmation of this model and elucidation of the precise molecular details of the mechanism will require further studies. The feasibility of introducing mutations into the cloned copy of the HA gene, of reinserting the mutant genes into SV40-HA vectors and testing their effects on fusion activity after expression in CV-1 cells will allow definition of specific amino acids which participate in the fusion reaction.

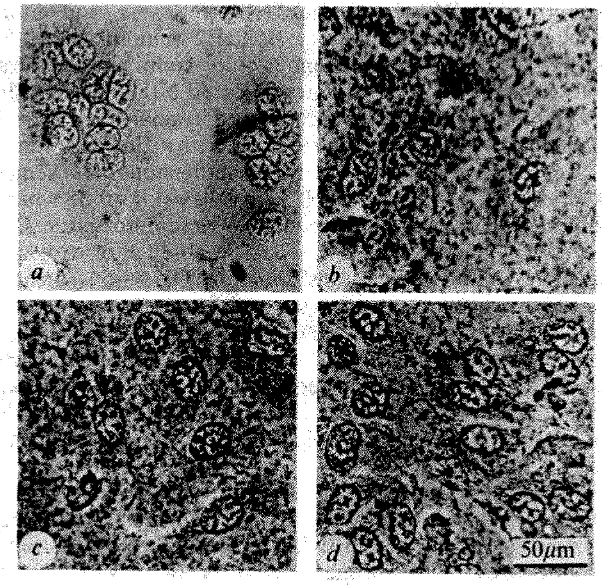

Fig. 2 Low *p*H-induced fusion of CV-1 cells expressing the HA of influenza virus. Confluent monolayers of CV-1 cells were infected with SVEHA3 as described previously[22]. 24 h post-infection, the cells were washed twice with serum-free Dulbecco's modified Eagle's medium (DMEM) and then incubated for 15 min at 37 °C in this medium (*c*, *d*) or medium supplemented with 10 μg ml^{-1} trypsin (*a*, *b*). The cells were then washed twice with DMEM plus 10% serum and then treated for 2 min at 37 °C with fusion medium (FM) (RPMI without bicarbonate, 0.2% bovine serum albumin, 10 mM MES, 10 mM HEPES) adjusted to either *p*H 5 (*a*, *c*) or *p*H 7 (*b*, *d*). These media were then replaced with DMEM containing 2% serum and the cells placed back at 37 °C for an additional 3 h. At this time they were fixed and stained with Giemsa as described previously[8]. Photomicrographs were taken with an Olympus PM-6 camera attached to an Olympus inverted phase microscope using Pan X film.

Finally, because of its binding and fusion capacities, the HA protein may be ideally suited to facilitate the efficient delivery of foreign substances into cells. Model studies aimed at developing liposome delivery methods can be carried out using cells expressing large amounts of HA from SV40-HA vectors. Preliminary results indicate that erythrocytes can be fused into such cells.

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Activation of a cellular transforming gene in tumours induced by Abelson murine leukaemia virus

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Abelson murine leukaemia virus (AbMuLV) is an acutely transforming retrovirus which induces lymphoid neoplasms in mice with latent periods of 3–5 weeks[1]. The oncogenicity of AbMuLV is attributed to expression of a viral transforming gene (v-*abl*) which encodes a protein with tyrosine-specific kinase activity[2]. Two recent findings, however, suggest that oncogenesis by AbMuLV may not be a single-step consequence of v-*abl* expression. First, Whitlock and Witte[3] observed that AbMuLV infection of bone marrow cells *in vitro* stimulated proliferation of immature blast cells which did not exhibit the *in vitro* growth properties or tumorigenicity characteristic of cells from AbMuLV-induced neoplasms. These AbMuLV-infected cell populations evolved to fully neoplastic cells only

after several weeks in culture, suggesting that secondary events were required for expression of the neoplastic phenotype[3]. Second, Grunwald *et al.*[4] reported the loss of the AbMuLV genome after prolonged *in vivo* passage of some AbMuLV-induced neoplasms, suggesting that expression of v-*abl* may be necessary for initiation, but not maintenance, of transformation. We report here that AbMuLV-induced neoplasms contain cellular transforming genes detectable by transfection which are distinct from AbMuLV sequences. Taken together, these results suggest that v-*abl* expression may induce early events in the neoplastic process and that secondary activation of a distinct cellular transforming gene may be involved in progression to neoplasia.

DNAs used as donors in transfection assays were extracted from two cell lines, B6T1E2 and B6T4E4, derived from early (fifth) *in vivo* passages of neoplasms induced by injection of newborn C57BL/6 mice with AbMuLV[4]. These tumour cell lines contain exogenously integrated AbMuLV DNA, express the v-*abl*-encoded transforming protein p160, and exhibit phenotypic markers (Thy-1-negative, Lyt-1-negative, Lyt-2 negative, immunoglobulin-negative, DNL1.9-positive[5] and 14.8-positive[6]) characteristic of AbMuLV-induced pre-B-lymphocytic neoplasms (ref. 4 and R. Risser, personal communication).

B6T1E2 and B6T4E4 DNAs induced transformation of NIH 3T3 cells with high efficiencies (approximately 1 transformant per μg DNA; Table 1). These transforming efficiencies are comparable to those obtained with DNAs of other human or mouse lymphoid neoplasms[7], but are at least 200-fold higher than the transforming efficiency of normal cell DNAs. Several foci of NIH cells transformed by each tumour DNA were grown to mass culture for further study. DNAs of these transformed NIH cells induced transformation in secondary transfection assays with efficiencies comparable to those obtained with the primary tumour DNAs (see Table 1). These results indicate that AbMuLV-induced neoplasms contain activated transforming genes which can be detected and serially passaged by transfection of NIH 3T3 cells.

The content of AbMuLV DNA in NIH cells transformed by these tumour DNAs was investigated by restriction endonuclease digestion and blot hybridization using a v-*abl*-specific fragment of a cloned AbMuLV provirus (λAM-1)[8] as probe. *Sac*I digestion of normal mouse DNA yielded three endogenous c-*abl*-containing fragments with molecular weights of 16, 5.8 and 3.0 kilobase (kb)[9] (Fig. 1). As *Sac*I cleaves within the AbMuLV long terminal repeat present at both ends of integrated viral DNA and at one internal site in the v-*abl* sequences[9], DNA of AbMuLV-infected cells (for example, B6T4E4 tumour cells) yielded two additional exogenous v-*abl*-containing *Sac*I fragments of 3.2 and 3.5 kb (Fig. 1*a*). Similar exogenous v-*abl* *Sac*I fragments were detected in B6T1E2 DNA (not shown). In contrast, DNAs of three NIH cell lines transformed by B6T4E4 tumour DNA (Fig. 1*c–e*) and of three NIH cell lines transformed by B6T1E2 tumour DNA (Fig. 1*f–h*) yielded only the endogenous c-*abl*-containing *Sac*I fragments detected in DNA of untransformed NIH 3T3 cells (Fig. 1*b*). These observations were confirmed by analysis of *Bam*HI-digested DNAs. NIH cells transformed by B6T4E4 tumour DNA (Fig. 1*k–m*) contained only the endogenous c-*abl* sequences present in DNA of NIH 3T3 cells[9] (Fig. 1*j*), whereas additional exogenous v-*abl* sequences were detected in DNA of B6T4E4 tumour cells (Fig. 1*i*). The absence of exogenous v-*abl* DNA in NIH cells transformed by DNAs of AbMuLV-induced tumours indicates that the transforming activity of these tumour DNAs is not mediated by transfer of the AbMuLV genome. As none of the six transformed NIH cell lines contained exogenous AbMuLV sequences, it seems that the transforming activity of AbMuLV DNA is significantly lower than that of the transforming gene detected in these transfection assays.

The transforming genes detected by transfection of AbMuLV-induced tumour DNAs were further characterized by investigating their sensitivity to cleavage with restriction endonucleases. Transforming activities of DNAs of both tumours studied were inactivated by digestion with either *Bam*HI or *Xho*I but not by digestion with *Eco*RI or *Hin*dIII (Table 2). In contrast, transforming activity of AbMuLV DNA is not affected by digestion with *Bam*HI[10]. The susceptibility of AbMuLV-induced tumour DNAs to restriction endonuclease digestion is the same as that previously reported for DNAs of four human pre-B-lymphocytic neoplasms, but differs from the patterns of inactivation observed for transforming activities of DNAs of human and mouse neoplasms representing intermediate and mature stages of B- and T-lymphocyte differentiation[7]. These results indicate that the transforming gene detected by transfection of DNAs of AbMuLV-induced mouse pre-B-lymphocyte neoplasms is closely related to the transforming gene activated in human pre-B-lymphocyte neoplasms.

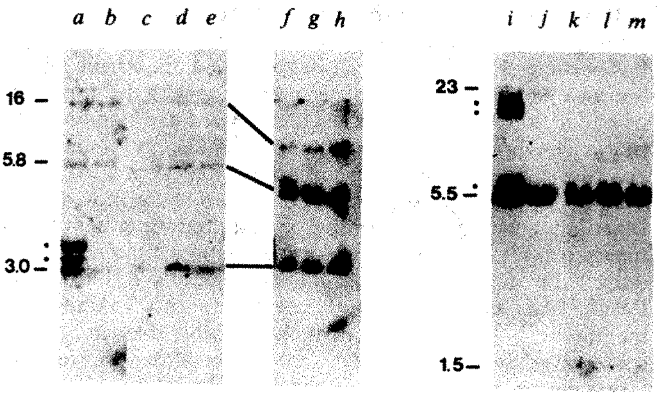

Fig. 1 Analysis of *abl* sequences in transformed NIH cells. Cellular DNAs were digested with either *Sac*I (*a–h*) or *Bam*HI (*i–m*), electrophoresed in agarose gels and analysed by Southern blot hybridization[18] as described previously[7] with ^{32}P-DNA probe prepared by nick-translation of a 1.5-kb v-*abl*-containing *Bgl*II fragment of λAM-1 (ref. 8). *a*, *i*, B6T4E4 DNA; *b*, *j*, NIH 3T3 DNA; *c–e*, *k–m*, DNAs of three independent lines of NIH cells transformed by B6T4E4 DNA; *f–h*, DNAs of three independent lines of NIH cells transformed by B6T1E2 DNA. The molecular weights of the endogenous normal mouse c-*abl* fragments[9] are indicated in kb. Dots indicate exogenous v-*abl* fragments in B6T4E4 DNA.

Table 1 Transforming activity of DNAs

Donor DNA	Total foci/total recipient cultures	Foci per μg DNA
AbMuLV-induced tumours:		
B6T4E4	321/14	1.1
B6T1E2	416/16	1.3
Transformed NIH cells:		
NIH (B6T4E4) 3-2	51/6	0.43
NIH (B6T4E4) 3-1	85/6	0.71
NIH (B6T4E4) 1-1	93/5	0.93
NIH (B6T1E2) 4-1	56/6	0.47
NIH (B6T1E2) 1-2	58/6	0.48
NIH (B6T1E2) 1-1	47/6	0.39
Controls:		
Normal mouse thymocytes	0/15	<0.003
Salmon sperm	6/100	0.003
Normal mouse liver	0/20	<0.003
NIH 3T3	0/25	<0.002

NIH 3T3 cells were exposed to 20 μg of donor DNA per recipient cell culture and foci of transformed cells were counted 11–13 days after transfection[7]. B6T4E4 and B6T1E2 are AbMuLV-induced mouse pre-B-lymphocyte neoplasm cell lines. Three independent foci of NIH cells transformed by each of these tumour DNAs (designated NIH (B6T4E4) and NIH (B6T1E2) cells) were grown to mass culture and used as donors of DNA in secondary transfection assays. Control DNAs were from normal mouse tissues, NIH 3T3 cells and salmon sperm.

Table 2 Digestion of tumour DNAs with restriction endonucleases

	Foci per μg DNA				
	Undigested	*Bam*HI	*Eco*RI	*Hin*dIII	*Xho*I
B6T4E4	1.0	0.01	1.1	1.2	0.06
B6T1E2	0.75	0.01	0.95	1.3	0.03

DNA (80 μg) of AbMuLV-induced tumours was digested to completion with the restriction endonucleases indicated. Bacteriophage λ DNA (2 μg) was included in a separate aliquot of each reaction that was incubated in parallel and analysed by electrophoresis in agarose gels to monitor the specificity and extent of digestion. Transforming activities of digested and undigested DNAs were assayed by focus formation on NIH 3T3 cells.

Previous studies have identified transforming genes in neoplasms induced by the weakly oncogenic retroviruses avian lymphoid leukosis virus (LLV), mouse mammary tumour virus (MMTV) and murine leukaemia virus (MuLV)[7,11,12]. In contrast to acute transforming retroviruses such as AbMuLV, these weakly oncogenic retroviruses do not contain specific viral transforming genes and require long latent periods (>6 months) for induction of neoplasia. The transforming genes detected by transfection of DNAs of LLV-induced chicken B-cell lymphomas and of MMTV-induced mouse mammary carcinomas were not linked to viral DNA sequences, indicating that oncogenesis by these viruses involves indirect activation of cellular transforming genes[11,12]. In addition, restriction endonuclease analysis indicated that the transforming genes activated in MMTV-induced mammary carcinomas, or in MuLV-induced lymphoid neoplasms, were also activated in chemically induced or spontaneously occurring neoplasms of the same cell types[7,12].

Induction of chicken B-cell lymphomas by LLV also involves activation of a cellular gene (c-*myc*) homologous to the transforming gene of the acute transforming virus MC-29, by adjacent integration of exogenous LLV transcriptional regulatory sequences[13]. However, the transforming gene detected by transfection of chicken B-cell lymphoma DNAs is not homologous to c-*myc*, indicating that LLV-induced B-cell lymphomas contain at least two distinct transforming genes[14]. Since induction of lymphomas by LLV seems to be a multi-step process, it has been suggested that the two distinct transforming genes may act at different stages of neoplastic development[14].

The present experiments suggest that oncogenesis by an acute transforming virus, AbMuLV, can also involve indirect activation of a cellular transforming gene. As only two tumours were analysed in these experiments, further studies will be required to assess the generality of the results. Together with the recent findings of Whitlock and Witte[3] and Grunwald *et al.*[4], however, our results suggest the possibility that the v-*abl* gene product acts at an early stage of oncogenesis and that progression to neoplasia involves activation of a distinct transforming gene detected by transfection. Rather than inducing transformation as a single-step event, the role of v-*abl* in the neoplastic process may be similar to that of the activated c-*myc* gene in LLV-induced chicken lymphomas.

Restriction endonuclease analysis of the transforming gene detected by transfection of DNA of AbMuLV-induced pre-B-lymphocyte neoplasms indicates that this gene is closely related to one previously detected in human neoplasms of the same cell type[7]. AbMuLV has also been reported to induce neoplasms of more mature B lymphocytes[15], T lymphocytes[16] and myeloid cells[17]. It will be of interest to characterize cellular transforming genes activated in AbMuLV-induced tumours of these other cell types in relation to the stage-specific transforming genes reported in other mouse and human B- and T-lymphocyte neoplasms[7].

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Cooperative deoxygenation of haemoglobin: asymmetry of binding and subunit differences

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Oxygen uptake by the blood in the lungs of a resting human brings the level of saturation of haemoglobin from ~70% to >97% (refs 1, 2). The recourse to this upper portion of the oxygen binding curve is reflected in an asymmetry of the isotherm which is frequently reported[3,4], but which has not been accorded any physiological role. This asymmetry is responsible for a more pronounced cooperativity of the deoxygenation at nearly complete saturation than that displayed in the typical sigmoidal pattern of loading at low oxygen tensions. Such behaviour is viewed here as a consequence of and a possible rationalization for the presence of two similar but distinct types of subunit in the haemoglobin tetramer.

A binding process would be defined as symmetrical if the fractional extent of saturation, θ minus 1/2, plotted against $\log(P/P_{1/2})$, where P is the oxygen tension and $P_{1/2}$ that corresponding to $\theta = 1/2$, yielded a curve with inversion symmetry about the origin. For ligation to a tetramer this symmetry condition would require[5] that $\sigma = (K_{2d}K_{3d})/(K_{1d})(K_{4d}) = 1$ where K_{1d}–K_{4d} are the intrinsic dissociation constants for the first to the fourth attached oxygen, respectively.

The first and last dissociation constants (K_{1d} and K_{4d}) are obtainable by extrapolation[3] as indicated in Fig. 1*a*,*b*. Greater uncertainty applies to the determination of K_{2d} and K_{3d} but their more reliably determined product[6] is sufficient, with K_{1d} and K_{4d}, to specify σ (designated W previously)[3,4]. Deviation of this parameter from unity has been adduced as evidence against several models which seek to express the four dissociation constants in terms of a smaller number of parameters[5,7,8].

From extant data for human haemoglobin stripped of diphosphoglycerate (DPG), a value of $\sigma = 29$ can be calculated[9], while $\sigma = 47$ for the same haemoglobin in the presence of DPG[10]. In contrast, a value of 84 has been reported for whole human blood[4]. Thus there is considerable variability in this parameter, due to the uncertainties in K_{2d} and K_{3d}, and because of differences in solution conditions and in measurement techniques. No values of $\sigma < 1$ have been reported and the phosphorylated co-factors[11,12], when present, elevate this parameter.

The relationship of σ to the pattern of oxygen ligation can be seen by comparing the rates of change of the slopes in Fig. 1. For the sigmoidal region at low oxygen tensions in Fig. 1*a*

$$[d^2\theta/d(P/K_{1d})^2]_{P\to 0}=2[3(K_{1d}/K_{2d})-4] \qquad (1)$$

while the comparable quantity for the dissociation curve in Fig. 1*b* is given by

$$[d^2(1-\theta)/d(K_{4d}/P)^2]_{P\to\infty}=2[3(K_{3d}/K_{4d})-4] \qquad (2)$$

The introduction of the factors K_{1d}^2 and K_{4d}^2 renders these derivatives independent of the choice of the standard state for the ligand. For a hyperbolic binding curve, as found with myoglobin or with independent binding and four identical intrinsic constants, both derivatives equal −2.

The ratio of the second derivative in equation (2) to that in equation (1) is greater than or equal to σ. It is equal only if $\sigma = 1$, in which case, changes of abscissae in the two graphs to P/K_{1d} and K_{4d}/P would make the curves superposable—another consequence of symmetry. With the cited values of σ, the sigmoidality of the dissociation curve in Fig. 1*b* as characterized by the second derivative can be 30–100 times greater than that shown in the customary binding curve of Fig. 1*a*. The asymptotic flattening in the latter provides no visual hint of the marked cooperativity of deoxygenation. Haemoglobin loads oxygen at a *p*H higher than it yields it up (Bohr effect), as indicated in Fig. 1*a*. The ratio of the sigmoidality parameters might be anticipated to be even greater at *p*H values lower than the slightly alkaline ones for which the σs quoted here apply.

A search for the origin of this asymmetry focuses naturally on the pairs of α- and β-subunits comprising the haemoglobin tetramer. A model can be advanced along the lines of those proposed previously[7] which allows for variations in reaction with oxygen of these two types of polypeptide chain in the tetramer. Such a model must minimally contain two intrinsic dissociation constants $K_{\alpha d}$ and $K_{\beta d}$ for the desorption of the first oxygen bound, respectively, to one α- and one β-subunit. Four interaction parameters, $\rho_{\alpha\alpha}$ and so on (see Fig. 2), measure the extent of diminution of dissociation from one haem site effected by oxygens occupying neighbouring sites. These quantities are expected to have an upper limit of unity with strong repression of dissociation characterized by magnitudes substantially less than one. They can be related directly to the conditional free energies of site occupancy[13]. Expressions for the four intrinsic dissociation constants involve all six of the above parameters. The asymmetry is given by

$$\sigma=[(K_{\alpha d}+K_{\beta d})^2\rho_{\alpha\alpha}\rho_{\beta\beta}]/[(K_{\alpha d}\rho_{\alpha\alpha})+(K_{\beta d}\rho_{\beta\beta})]^2 \qquad (3)$$

The cross-interaction parameters $\rho_{\alpha\beta}$ and $\rho'_{\alpha\beta}$, of which two were introduced because each subunit is differently disposed structurally in the tetramer with respect to its two dissimilar neighbours[14], are absent from equation (3). This is because their magnitudes, which affect equally both α and β, cannot contribute to the asymmetry. From equation (3) $\sigma = 1$ if $\rho_{\alpha\alpha} = \rho_{\beta\beta}$ or $K_{\alpha d}\rho_{\alpha\alpha}^2 = K_{\beta d}\rho_{\beta\beta}^2$.

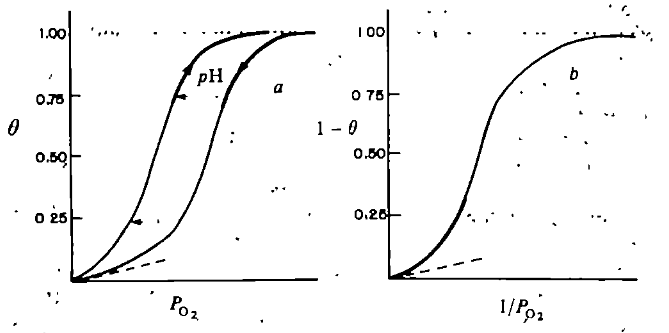

Fig. 1 *a*, Customary curves for oxygen loading (left) and unloading (right) by haemoglobin. The darker portions of the curves indicate the operative region in resting humans. The broken line is the initial tangent given by $[d\theta/dP]_{P\to 0}=1/K_{1d}$. *b*, Fractional extent of unsaturation plotted against $1/P$ to illustrate the highly cooperative deoxygenation reaction. The limiting tangent at high oxygen tension as shown by the dashed line is $[d(1-\theta)/d(1/P)]_{P\to\infty}=K_{4d}$.

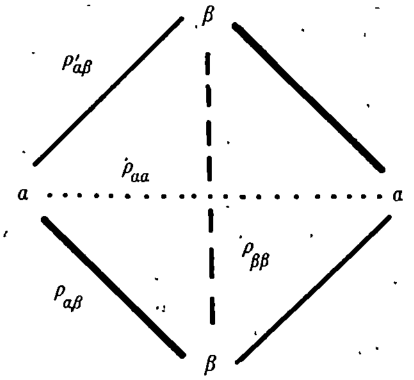

Fig. 2 The haemoglobin tetramer with six edges marked by parameters for repression of dissociation induced by intersubunit contacts. They are formally represented as two site–site interactions between like neighbours ($\alpha\alpha$ and $\beta\beta$) and two ($\alpha\beta$ and $\alpha\beta'$) pairs of interactions between unlike partners.

The condition of practical interest of $\sigma > 1$ can be shown to mandate either of two pairs of inequalities, that is, $\rho_{\alpha\alpha}/\rho_{\beta\beta} > \sigma \simeq$ 30–90 and $K_{\beta d}/K_{\alpha d} > \sigma^{1/2} \simeq 5$–9, or the opposite obtained by interchanging α and β. Site–site as opposed to single site interaction accounts for the higher power of σ appearing in the first inequality. This is the experimentally favoured pair as it requires that the β-chains, which self-aggregate and are in more intimate contact[14] in haemoglobin A, are the more efficacious in depressing the dissociation constants. Also, the companion inequality is consistent with reports[15–17] that the α-chains are the first to bind oxygen.

While intense physical activity can cause blood in the pulmonary capillary bed to be more deprived of oxygen[1] than the 70% level cited above, the usual oxygen tension must convert it overwhelmingly (and rather rapidly[18]) to quite high extents of saturation. Thus the cooperative character to the oxygenation must be of secondary importance to that exhibited by the deoxygenation in states of physiological stress. The asymmetry of the ligation clearly serves this purpose. An asymmetry of reaction assignable to a structural cause is comprehensible. However, the existence of two kinds of subunit in haemoglobin is of wide occurrence, even among primitive vertebrates. Is its origin as well as its preservation attributable to the need for these diverse organisms to maintain a capacity for an asymmetric response to oxygen requirements?

Many of the conclusions about the quantitative behaviour of haemoglobin have been reached independently by Dr Gregorio Weber through consideration of the general consequences of asymmetric ligation. I thank Drs John Severinghaus and Peter Torgerson for helpful comments and the NSF for support (grant PCM-8022006).

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BOOK REVIEWS

A philosopher among the physicists

Donald MacKay

Postscript to the Logic of Scientific Discovery, Vols II and III. By Karl Popper, edited by W.W. Bartley, III. Vol. II *The Open Universe: An Argument for Indeterminism.* Pp.185. ISBN 0-09-146180-4. Vol. III *Quantum Theory and the Schism in Physics.* Pp. 229. ISBN 0-09-146170-7. (Hutchinson/Rowman & Littlefield: 1982.) Each volume £15, $24.50. Vol. I, *Realism and the Aim of Science,* has just been published in the United States and will appear in Britain early next year.

Sir Karl Popper is not the kind of original thinker who is content to swim peacefully against the stream of contemporary fashion. Instead, he takes Puckish delight in splashing the water in the faces of those who drift unthinkingly by. "Many younger physicists", he laments, "have grown up in a period of over-specialisation, and in the newly developing tradition of the cult of narrowness, and the contempt for the non-specialist older generation . . . a tradition which may easily lead to the end of science and its replacement by technology". He sees the current scientific orthodoxy as riddled with philosophical errors and confusions that urgently need sorting out; but where a lesser mind might be moved to attack these with grim and tedious analyses in abstract terms, it is Popper's genius to make the issues come alive in terms of actual events and concrete problems in the history of science, as seen by the people who faced them. The result is extraordinarily stimulating, whether or not one agrees with him. To come to grips with his thinking is to have one's mental muscles enjoyably stretched, and one's presuppositions submitted to searching scrutiny in an almost playfully challenging — though deadly serious — spirit.

These two volumes are part of a trilogy based on Popper's legendary *Postscript to The Logic of Scientific Discovery,* which after circulating privately for 25 years has now acquired a variety of qualifying and amplifying addenda. In Vol. II, *The Open Universe,* Popper is concerned mainly with refuting the idea that the world is deterministic in any of several senses which he explores. Three addenda argue for the reality of worlds other than the physical; and against ontological reductionism. Volume III, *Quantum Theory and the Schism in Physics,* is a hard-hitting attack on the subjectivist interpretation of quantum mechanics favoured by the Copenhagen School, and on what Popper sees as kindred interpretations of the Second Law of Thermodynamics. It includes a detailed argument for his well-known explication* of probability as "objective propensity", and ends with a "Metaphysical Epilogue" on the nature of science and its open problems, as seen in terms of his objectivist propensity interpretation.

What invigorates in Poppor is his robust and commonsensical realism. "Realism", he declares, "is linked with rationalism, with the reality of the human mind, of human creativity, and of human suffering". He scorns claims such as Wigner's that the laws of quantum mechanics cannot be formulated without recourse to the concept of consciousness, as

> based on bad philosophy and on a few very simple mistakes [which] I hope will soon be forgotten, while the great physicists who happened to commit them will be forever remembered by their marvellous contributions to physics: contributions of a significance and depth to which no philosopher can aspire.

He rebuts the idea that von Neumann conclusively proved the impossibility of "hidden variables"; and he effectively demolishes much subjectivist nonsense talked about the famous "Reduction of the wave packet". No teacher of quantum theory should miss the opportunity to sharpen his thinking against this abrasive whetstone, especially if he wants his pupils to distinguish solid science from fashionable dogma.

In his Epilogue Popper strikes an optimistic note, suggesting that by replacing talk of "particles" by talk of "propensities" as the objectively real elements of the physical theory of matter, the wave–particle dualism of orthodox theory could be dispensed with, and the dispute between Einstein's and Born's interpretations resolved. Particles, he tells us, "can be produced by propensities, and . . . up to a point . . . *are* propensities" (Vol. III, p. 196). One might have expected propensities to be linked more closely with events than with entities — with particle-impact and the like, rather than with particles; but Popper evidently has in mind Dirac's theory of the positron as an analogous line of thought (Vol. III, p.197). The Epilogue unfortunately does not go into enough detail to allow the fruitfulness of his suggestion to be assessed.

In his hot pursuit of subjectivity, there are times when Popper's polemical zeal drives him to cut some corners. Against subjectivist notions of entropy, for example, he offers as an objective criterion of disorder in a system the "chancelikeness" of the distribution of its molecules (Vol. III, p.112). Obvious counter-examples spring to mind: If a (highly-ordered) busload of soldiers scatter to precisely specified but randomly distributed hiding places, are they now any less orderly? Is the sequence of digits down the columns of a telephone directory "disorderly"? Compactness is only one form of order; and dispersion is not the same concept as disorder. Everything here depends on the data that determine the distribution of the dispersed members. To claim that Boltzmann's entropy-measure is *relative* to a specific data-base does not make it subjective; and the link between disorder and lack of information cannot be dismissed quite as easily as Popper suggests.

A passionate concern for human freedom and dignity shines through Popper's polemics against determinism, which he sees as incompatible with responsible choice and human creativity. He argues convincingly that deterministic concepts of our world have no scientific support; and he sets out in improved form his famous 1950 proof that no discrete-state computing machine could predict precisely the future of a universe that included itself, even in terms of the laws of classical (pre-Heisenberg) physics.

Even if we accept all this, however, and thoroughly sympathize with its motivation, some nagging doubts remain. First, as Popper himself acknowledges, our inability to predict a future event is neither a necessary nor a sufficient condition of its being undetermined; so his heavy emphasis on the unpredictability-to-us of the future can here be misleading and even counterproductive. Secondly, indeterminism would be of value in this connection only if it allowed scope for the control of human actions by human conscious choices; but for this Popper can only ask us to picture the mind (in a non-physical "World 2") as "interacting" with the brain (in "World 1"), without offering any scientific account of how such interaction could take place, let alone how his conjecture could be falsified. (His more extensive treatment of the topic with Sir John Eccles in *The Self and its Brain* (Springer-Verlag, 1977) still leaves these matters unclear.)

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*For Popper the "probability" of a physical event denotes an objective physical property of the generating situation, which he terms its "propensity" to produce the event.

My main doubt, however, is whether Popper has shown human freedom and creativity to *require* the brain to be physically indeterminate in its workings. He rejects — for good reasons — the simple equation of mind with brain; but he ignores another option which, as I see it, would safeguard all the values he wants to maintain without the awkward implications of interactionism. This would see mental activity, rather than *interacting with* the brain, as *embodied in* brain activity, in something of the sense in which an equation being solved by a computer is embodied in the computer's physical structure at the time. To say that the behaviour of a computer is determined by the equation it is solving, is quite compatible with saying that its physical workings are determined by the laws of physics. Indeed, from the programmer's standpoint, the latter may be a necessary condition of his claim; and there is certainly no need to prove the computer open to "non-physical influences from another world" in order to sustain that claim.

Courtesy Hutchinson

Karl Popper — "extraordinarily stimulating, whether or not one agrees with him".

Scientific determinism (in Popper's sense) may well be false and baseless; but for his elaborate attack on it to be of much consequence for human freedom he needs to show why the physical determinateness of brain processes would preclude the capacity of human beings to determine their actions freely in accordance with their conscious thinking, evaluating and choosing. As I have argued elsewhere — see *Mind* **69**, 31-40 (1960), and *Human Science and Human Dignity* (Hodder and Stoughton, 1979) — even if the outcome of such choosing were predictable by non-participant observers, it would not logically follow that the outcome was inevitable for the chooser himself, nor that *he* had really no options open to him; for this, too, is an objectively relativistic situation. What the non-participants would be correct to believe about the immediate future of the chooser's cognitive mechanism is something the chooser himself would in general be mistaken to accept as accurate and inevitable-for-him, since the physical changes required for its embodiment in his brain cannot but significantly affect its accuracy. Thus even the complete success of a prediction kept secret from the chooser until after the event would not show in retrospect that *he* had no other option open to him; for no prediction of the outcome existed, even unknown to him, that had an unconditional claim to *his* assent. There is, after all, nothing particularly shocking in the thought that the form of a future event may be fixed-and-inevitable relative to someone not causally coupled to it, while still indeterminate and open to the individual whose thinking and deciding will constitute the event in question.

This relativistic point bears particularly on Popper's dispute with Einstein over the implications of the latter's deterministic world-model, as narrated in Vol. II, pp.89-92. According to Popper, Einstein's views in 1950 implied that the future is contained in the past "as the chick in its egg". This, Popper argued, made the future "superfluous", and our sense of time "illusory". Moreover, "if we were experiencing successive [motion picture] shots of an unchanging world, then one thing at least was genuinely changing . . . our conscious experience" (Vol. II, p.92), and this "looked very much like a flat contradiction". As soon as we distinguish the different standpoints here, however, all appearance of contradiction vanishes. From the standpoint of the characters in a film (or a history book) their future is asymmetrical with their past in that, willy nilly, what they think and desire and do will play a part in determining its form. Their experience of change is not an illusion, but a fact of their world that they would be lying to deny. From the standpoint of the film-projectionist (or the book-reader), of course, the future of the characters is as much a fixed and unchanging fact-for-him as their past. It is "all in the book". To regard these views as logically conflicting, however, is surely a mistake; for the data available to the projectionist (or the bookreader), so far from showing the experience of the characters to be "illusory", actually supply confirmatory evidence of the changes experienced in their world, and of the asymmetry-to-them of past and future. It is thus rather touching to be told that the ageing Einstein, when confronted with Popper's arguments, merely "said that he was impressed by them, and that he had no answer for them" (p.92).

A reviewer's task is to be critical where he must; and indeed only lack of space precludes comment on a number of other matters, such as Popper's interpretation of the quantum-mechanical ψ-function, his tendency to confound rationality and rationalism, and his inclination to take "World 1" (the world of physical objects) as the "standard example of reality". Let me conclude, however, by warmly recommending the catalytic experience of reading — and re-reading — these mature and scholarly reflections of a great mind, on matters of central concern to physical science and of importance to us all. ☐

Donald MacKay, a physicist, is Emeritus Professor of Communication and Neuroscience at Keele University.

Digging deep into plasma physics

T.J.M. Boyd

The Interaction of Strong Electromagnetic Fields with Plasmas. By I.R. Gekker. Pp.324. ISBN 0-19-851467-0. (Oxford University Press: 1982.) £35, $85.

The coming of age of plasma physics has been reflected in its literature by a steady growth in the output of monographs as compared with the primers of years past. There has appeared lately a stream of more or less specialized works addressing topical — occasionally, by the time the books appear, not-so-topical — aspects of what is now a wide-ranging subject. Professor Gekker's book on the interaction of strong electromagnetic radiation with plasmas is avowedly specialist, and its content undeniably topical.

The text is in two parts. Part I summarizes the simple linear physics of plasma wave propagation, with a short excursion into electromagnetic wave absorption in inhomogeneous plasmas which serves as an introduction to the theme of the work proper. Part II consists of six chapters devoted to aspects of the nonlinear physics of propagation and absorption of electromagnetic waves, a subject to which Professor Gekker has himself contributed widely. Starting with a statement of the properties of nonlinear phenomena in plasmas — at four pages a *précis* of almost unbecoming brevity — Part II goes on to develop the interaction physics of charged particles with high frequency fields in both magnetized and unmagnetized plasmas. This is followed by a chapter on the interaction of strong electromagnetic fields with dense plasmas, altogether too ambitious a title in view of its very restricted compass. The final two chapters on the anomalous absorption of electromagnetic radiation and concomitant plasma heating are, by contrast, full and detailed and make up almost half the text. The book concludes with an extensive bibliography and a less than comprehensive index.

The work is given a distinctive flavour by its emphasis not only on the experimental aspects of the interaction of radiation with plasmas but on details of the experimental procedures themselves. While there are undeniably circumstances in which this amount of detail is valuable — even essential — one is left with a clear impression that on many occasions the descriptions provided serve no purpose and could have been dispensed with to advantage. Worse, however, is that they sometimes positively get in the way of an otherwise informative discussion. Examples of such over-indulgence are to be found in virtually every section of Part II. Some read like paraphrases of the original literature; others are probably relics from the lectures on which the book is based and would have been best left out. Had Professor Gekker been less prodigal in his attention to descriptive detail he might have felt able to devote correspondingly more space to discussing the implications of the various experiments for nonlinear plasma physics.

Another shortcoming, perhaps not unrelated to the book's origins in a course of lectures, is some tendency to be comprehensive rather than critical. While it is informative to have one experiment compared and contrasted with another, there is perhaps less merit in setting out a catalogue of work done without critical commentary. Another aspect of the same problem manifests itself in the lists of authors which are interspersed in the text, extending in one instance to 12 lines of unnecessary interruption. More substantial than these pinpricks is the considerable imbalance in the treatment of the subject matter. For example Chapter 9 forms almost one-third of the text, with one section alone (Section 9.5 on microwave absorption experiments) extending to 35 pages. This could have been subdivided to advantage.

These various criticisms should not be taken to imply that the book is without merit. It is a veritable mine of information — albeit one in which much of the ore is deep level — and will repay careful reading. The readership is likely to consist in large part of scientists active in this area of plasma physics. Unlike the original Russian version this translation is less likely to be used as a text in postgraduate courses though it could, and should, find a place on reading lists. ☐

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Abscission since Newton

Roy Sexton

Abscission. By Fredrick T. Addicott with illustrations by Alice B. Addicott. Pp.369. ISBN 0-520-04288-3. (University of California Press: 1982.) £29.75, $51.35.

LIKE MOST abscission physiologists I am occasionally confronted by some hearty soul who tells me that we would probably know more about abscission if only Newton had confined his thoughts to the processes that occurred just prior to the fall of the apple. The implication that there are major gaps in our knowledge of abscission is well founded but unfortunately these problems have failed to attract the attention they merit from botanists.

In the preface to *Abscission*, F.T. Addicott makes plain his aim to interest plant scientists from a wide range of backgrounds. Unlike most previous reviewers, he has not confined his attention to the physiology and agronomic importance of the process but has expanded the scope to include the lower plants and topics such as the genetics and ecology of abscission. During his 40-year association with the subject the author has accumulated 2,500 papers concerned with abscission and as a result he is able to write with unrivalled authority on most aspects of the process. The obvious zeal with which this information has been gathered is impressive, though great care has been taken not to overwhelm the reader and the information is presented in a concise yet readable form. There is little doubt that the massive effort involved in mastering this material has been worthwhile and will ensure that this volume will become the standard reference work for many years to come.

The first section of the book describes the various structures that can be shed from the plant, ranging in size from the smallest trichomes to the complete aerial portion of tumble weeds. Detailed accounts are given of the anatomy, cell biology and biochemistry of the weakening process. The section on the hormonal control of abscission is briefer than I had anticipated, and although the literature coverage is adequate it is not as comprehensive as that in other chapters. This section contains a spirited defence of the view that abscisic acid is a major controlling factor in abscission, which is perhaps what one would expect from a member of the team involved in the original characterization of the substance.

Also included are a chapter describing the increasing agronomic importance of manipulating abscission and another concerned with the ecological significance of different abscission strategies. The sections on the genetics and evolution of abscission are especially noteworthy because they represent the first serious attempts to review these subjects.

The front cover of the book carries not only the author's name but also that of his wife, Alice B. Addicott, who was responsible for the fine botanical drawings. This pleasant feature draws attention to the unrecognized yet important role that illustrators play in improving the clarity of scientific texts. However I appreciate mention of her name for another reason. I was once asked by a student why the recognized abbreviation for abscisic acid was ABA (see *Science* **159**, 1493; 1968) and not AbA which seemed more logical. Is this perhaps just a coincidence; or is there another explanation? ☐

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Variety in singular behaviour

N.B. Davies

The Shelduck: A Study in Behavioural Ecology. By I.J. Patterson. Pp.276. ISBN 0-521-24646-6. (Cambridge University Press: 1982.) £27.50.

THE CURRENT trend for research in behavioural ecology is first to think of an interesting idea and then of a species that will be good for testing it. Detailed studies of all the various aspects of a single species have become less fashionable and are sometimes disparagingly labelled "the aardvark approach"; if no one has yet studied aardvarks then we use this as the main excuse for doing research on them. This view is unfortunate because we have only to think of Lack's robins, Nice's sparrows and Tinbergen's gulls to remind us that some of the main ideas in behaviour and ecology have been derived from long-term studies of particular species.

Shelducks have been studied on the River Ythan in Scotland for the past 20 years by Ian Patterson and students from Aberdeen University, forming the subject of six theses and over 20 published papers. This book, which summarizes the work and relates it to other studies, is a good example both of how general theories can best be tested with long-term data and also of how a detailed study of one species can give rise to new ideas of wider interest. The book is organized around the shelduck's annual cycle, beginning with the extraordinary autumn migration to the mudflats of north-west Europe where, safe from predators, thousands of birds congregate to moult, dropping all their flight feathers

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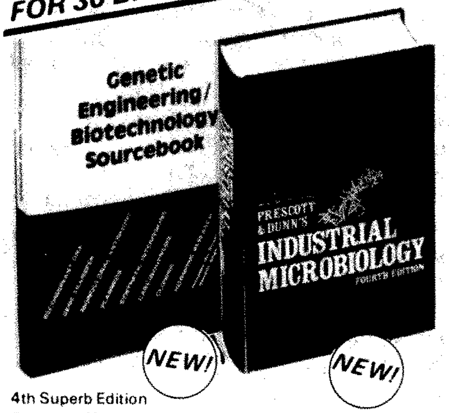

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simultaneously and so becoming flightless for about a month. Then there follow chapters on winter flocking, territories, nest sites, laying and incubation, care of the young and population regulation.

The first workers in the 1960s interpreted much of the shelduck's social behaviour and population dynamics in relation to Wynne-Edwards's theory of reproductive restraint by individuals in order to prevent over-population. The groups of birds which assembled in the sand dunes in spring were regarded as "parliaments", where decisions were made for the good of the group as to who should breed and who should be excluded. Patterson's studies have shown that this behaviour makes much better sense from a selfish-individual point of view. The non-breeders turn out to be mainly young birds prospecting for nest burrows late in the season, probably to gain familiarity with the breeding area for the next year. The parliaments appear to involve subordinate or previously unsuccessful birds following dominants around to parasitize their knowledge of nest sites. There is also the possibility that "non-breeders" in fact dump eggs in the breeders' nests; up to half of the nests in some years contain eggs laid by more than one female. Shelducks are so shy, however, and their nests at the ends of rabbit burrows are so inaccessible, that the details of these multiple clutches are still unknown.

Equally fascinating is the phenomenon of brood mixing. When parents escort their ducklings from their burrows in the dunes to feeding grounds on the estuary, they often encounter other families. During the aggressive encounters between adults, ducklings often attach themselves to the wrong parents. Some pairs may lose all their young while others accumulate large crèches. The data on duckling survival show no benefit to the young from these associations and measurements of adult survival suggest likewise no benefit to either donor or recipient parents. Patterson suggests therefore that brood mixing is not adaptive and occurs when shelducks breed at unusually high densities. Fervent adaptationists will probably want more data before they are convinced, but critics of the so-called adaptationist programme will perhaps be relieved to find that animals sometimes simply make mistakes!

One of the main messages which comes out of this excellent book, and from other long-term studies like it, is that there are not only marked differences between populations but also between years within the same population. Such variability makes field work all the more interesting, but it means that we need to be careful before we generalize from the findings of short-term research projects. ☐

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The planets exposed

Dennis L. Hartmann

The Cambridge Photographic Atlas of the Planets. By Geoffrey Briggs and Frederick W. Taylor. Pp.255. ISBN 0-521-23976-1. (Cambridge Unversity Press: 1982.) £12.50, $24.95.

We are nearing the end of a remarkable period of exploration of the Solar System. Six of the nine planets and their satellites have been the objects of close scrutiny by unmanned probes; pictures and data have been returned to Earth from the surfaces of our nearest planetary neighbours; men have walked on the Moon. All of this has taken place in the past 20 years, most of it in the past decade.

The early symbol of a new public awareness of the Solar System and our place in it was perhaps the sight of Earth from the vicinity of the Moon obtained by the Apollo astronauts. In *The Cambridge Photographic Atlas of the Planets* this perspective on our familiar planet and its satellite is supplemented by a large number of close-up photographs and other images of five additional planets and most of their satellites. It is this new view of the Solar System, obtained mostly from unmanned spacecraft, which Briggs and Taylor present in their book.

The authors begin with an overview of current ideas regarding the origin and subsequent evolution of the Solar System, directing particular attention towards the formation of the planets and the processes acting to determine the properties of their surfaces and atmospheres. This account provides the background for the six main chapters in which the planets are discussed in order of increasing distance from the Sun, beginning with the rather Moon-like planet Mercury and concluding with the intricate and still somewhat mysterious Saturnian System.

Each chapter opens with a section of text, first describing the overall characteristics of the planet and then delving into the scientific questions they present. Observations obtained by remote probes have provided many clues about the history and current state of the interiors, surfaces and atmospheres of the planets and their satellites, and these new findings are outlined both readably and authoritatively. Following the text comes a section of photographs and maps showing the surface or atmosphere of the planet in question and those of its satellites. The pictures, about half of them reproduced in colour, include some of the most spectacular and beautiful available, but many have also been chosen because they illustrate some interesting feature or provide some important clue about the body in question. A careful reading of the text heightens one's interest in the pictures, but lucid yet detailed captions mean that it is not necessary to read the text to appreciate the significance of the illustrations.

The fascinating observations of the planets and their satellites illustrated and discussed in this book are likely to provoke the interest even of those with no previous exposure to planetary science. One can ponder such questions as whether there are continents on Venus and how the peculiar patterns in the clouds of its atmosphere are maintained. The surface features of Mars, the circulation of the atmosphere of Jupiter and the rings of Saturn provide equally challenging opportunities for inquiry and debate. Some readers, however, will be disappointed that no bibliography or source list is included to enable them to probe such issues more deeply.

Geoffrey Briggs and Frederick Taylor have a long-standing involvement in planetary exploration and write clearly and engagingly about their subject. Their book is likely to please a wide range of readers. The text should be comprehensible to the general reader, though it will be better understood and appreciated by those with some knowledge of the physical sciences. Because of its remarkable combination of strong visual appeal and scientific interest, *The Cambridge Photographic Atlas of the Planets* will be equally at home on the coffee table and in the research library.

Dennis L. Hartmann is an Assistant Professor of Atmospheric Sciences at the University of Washington, Seattle.

Wooden characters brought to book

Peter D. Moore

Identification of Modern and Tertiary Woods. By A.C. Barefoot and Frank W. Hankins. Pp.220. ISBN 0-19-854378-6. (Oxford University Press: 1982.) £47.50, $98.

FROM the title of this book, one might be forgiven for thinking that the volume will provide a means for the identification of woods, both Tertiary and modern; in fact it does not. It sets out to illustrate those features of wood anatomy which can most usefully be applied to the characterization and identification of timber. It is, therefore, a guide for both students and timber technologists which will enable them to use with greater confidence some of the multiple-entry card keys currently on the market. Much of the book is actually based upon one of these keys, available on microfiche from Harvard University, which is itself a compilation of earlier keys and is still incomplete at present.

The opening chapter asks the question, "Why identify and study wood?", but fails adequately to answer it. It is in any case a rather superfluous question since the reader presumably has his own reasons, but a survey of these reasons could be of general interest.

There follows an introductory chapter on wood anatomy, preceding the more detailed analysis of key features used in wood identification. This account lacks structure in the way it deals with topics and fails adequately to define certain terms. Even such a straightforward expressions as "bark" are not clearly defined in the text and the drawings are sometimes so dense that the lines from labels are lost in them. More subtle terms, such as "roey-grained", are used but not explained. One redeeming feature is the use of some excellent scanning electron micrographs, a technique which could have been further exploited and improved by detailed labelling — one photograph has letters attached but lacks an explanation in the legend. As an introduction to wood anatomy, one wonders whether this chapter is indeed aimed at the newcomer or, more likely, the experienced botanist.

The main body of the book explains each of the 86 wood characters used in the punch card key, sometimes a difficult task, especially when such characters as "late wood conspicuous" need to be interpreted. On the whole, this section of the book is very helpful, especially in its profuse use of high-quality micrographs and detailed line drawings; indeed, it is hard to imagine how the key could be tackled without its aid. Finally, the arrangement of the card key is explained and a timely caveat is given concerning the variability of wood within species as a consequence of both geographical range differences and variation in environmental factors.

Taken as an illustrated adjunct to the punch card key, this volume is an extremely valuable asset. It should not, however, be regarded as a textbook of wood anatomy or as an identification manual in its own right. □

Peter D. Moore is Senior Lecturer in the Department of Plant Sciences, King's College, University of London.

- Elaine Morgan's *The Aquatic Ape: A Theory of Human Evolution* (reviewed in *Nature* **296**, 511; 1982) is now available in the United States. The book is published by Stein & Day, and costs $14.95.
- The second volume of *Cell Biology of Physarum and Didymium*, edited by Henry C. Aldrich and John W. Daniel, has recently been published by Academic Press. For a review of Vol. 1 (*Organisms, Nucleus, and Cell Cycle*) see *Nature* **298**, 874; 1982. The second volume, entitled *Differentiation, Metabolism, and Methodology* costs $52, £??.

The noise of music

H.C. Longuet-Higgins

Music, Mind, and Brain: The Neuropsychology of Music. Edited by Manfred Clynes. Pp.430. ISBN 0-306-40908-9. (Plenum: 1982.) $39.50, £24.89.

THE literature of music psychology — and *Music, Mind, and Brain* is no exception — deals with a wide variety of topics ranging from the neurophysiology of pitch perception to the affective responses of listeners. In order to bring some semblance of order to this vast area of inquiry it would therefore seem essential for anyone writing about the psychology of music to distinguish clearly at the outset between auditory stimuli in general and music in particular. The most interesting questions in the subject surely have to do with the differences between our responses to different pieces of music, but without a clear definition of what counts as a piece of music it is impossible to formulate such questions precisely. If music were simply — as Charles Lamb once complained — "the noise that they make on purpose", then folk tunes, electronic squawks, animal noises and so on would have to be treated on an equal footing by the psychologist of music.

It seems to me that what distinguishes music from non-music is how we conceptualize it: a piece of music is a composition of notes, linked together by rhythmic and tonal relations, just as a sentence is a linguistic structure composed of words which are connected by syntactic and semantic relations. Modern theoretical linguistics became a science when this perspective finally came into focus, and thanks to the work of Chomsky and his successors it is now possible to see that the central problems of language have to do with the systematic relations between the detailed structures of utterances and the messages which they serve to convey. No psycholinguist would now dare to publish a paper which treated utterances or texts as unstructured sequences of noises or marks, and one might have hoped that an equivalent distinction would be taken for granted by psychologists of music. But with one or two laudable exceptions, the authors of *Music, Mind, and Brain* seem quite unaware of the need to explain, when they use the word "music", exactly what they are talking about.

The book contains 21 chapters, together with a preface, a foreword and some appendices; a short sound sheet is tucked into the back of the book. The great majority of the contributions consist entirely of words, with no musical quotations; of the remainder only a handful make any attempt to describe in detail the rhythmic or tonal structures of the musical examples. In the first three chapters, for instance, Minsky, Pribram and Roederer discourse upon musical cognition, the mechanisms of auditory memory and other broad questions, but are lamentably vague about music itself. In two other chapters Clynes and his colleagues discuss the "essentic forms" of seven basic emotions, and the characteristic "pulse" by which a composer, they claim, hall-marks his compositions; but they fail to offer any empirical tests by which this claim could in principle be falsified.

Chapter 5, by Jackendoff and Lerdahl, is possibly the best in the book. These authors clearly recognize the central importance of structural concepts in the description of music, but even they omit to give any precise account of tonal relations, or to explain, for instance, the relation between the time signature of a classical composition and rhythmic structures of the individual bars. Of the remaining contributions, the most interesting are those of Diana Deutsch on the concept of Gestalt in music, of Sundberg on the expression of emotion in singing, and of Makeig on the theory of tonality. Makeig is familiar with the current theory of tonal relations but seems to be unaware of the insights expressed by the late Deryck Cooke in his classic book *The Language of Music* (Oxford University Press, 1959).

Elsewhere, Balzano offers a group-theoretical account of "pitch sets", and rediscovers the three-dimensional tonal space first proposed by Longuet-Higgins in 1962, but does not illustrate his proposals with any interesting musical examples. And Terhardt, an acknowledged authority on auditory pitch perception, proposes a musically outrageous "root sequence" for the first few bars of *Tristan und Isolde*, in which the augmented 4th (F to B) in the famous chord is implicitly misrepresented as a diminished 5th (E sharp to B). In all it seems that some neuropsychologists of music have much to learn before their ideas need to be taken very seriously by competent musicians. ☐

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Pascal's mind and Pascal's science

A.W.F. Edwards

Pascal: Adversary and Advocate. By Robert J. Nelson. Pp.286. ISBN 0-674-65615-6. (Harvard University Press: 1982.) $22.50, £18.

BLAISE Pascal was born in 1623 in Clermont, France. In 1631 his father Etienne Pascal (himself an able mathematician who gave his name to the "limaçon of Pascal") moved to Paris in order to secure his son a better education. There, Etienne was one of the founders of Marin Mersenne's "Academy", the finest exchange of mathematical information in Europe at the time. To this informal academy he introduced his son at the age of 14, and Blaise immediately put his new source of knowledge to good use, producing (at the age of 16) his *Essay pour les Coniques* based on the work of Desargues, another founder-member of the Academy.

In the succeeding years the young Pascal designed and had built the first mechanical adding machine and conducted experiments into the nature of a vacuum, but his chief scientific contribution was to lay the foundations of the theory of probability.

The fame of Pascal, however, rests only to a small degree on his scientific work. Both adversary and advocate, as the title of this book suggests, he employed his exceptional talents in the public pursuit of religious controversy (especially through the *Lettres Provinciales*) and the compilation of extensive notes (the *Pensées*) intended as the basis of *An Apology for the Christian Religion* which he did not live to complete. These works not only provide extraordinary insights into the human condition, but have had a great and lasting influence on French language and literature.

Yet his influence on probability was scarcely less; before his time, probability calculations amounted to no more than the enumeration of equally-probable outcomes in games of chance. Pascal introduced the important idea of manipulating expectations using recursively the fact that if expectations of gain X and Y are equally probable, the expectation is $\frac{1}{2}(X+Y)$. He also introduced the binomial distribution for equal chances and with its help, and that of mathematical induction applied to expectations, solved the Problem of Points for two players.

This problem was the topic of the correspondence between Pascal and Pierre de Fermat in the summer of 1654 which, together with Pascal's contemporary *Traité du Triangle Arithmétique*, includes not one but three solutions. Suppose two players stake equal money on being the first to win n points in a game in which the winner of each point is determined by the toss of a coin, heads for one player and tails for the other. If such a game is interrupted when one player still lacks a points and the other b, how should the stakes be divided between them?

On the evidence of the correspondence, Fermat and Pascal independently concluded that the problem could be solved by noting that at most $(a+b-1)$ more tosses will settle the game, and that if this number of tosses is imagined to have been made, the resulting 2^{a+b-1} possible games (each equally probable) may be classified according to the winner in each case, the stakes then being divided accordingly. Thus the real game, of indeterminate length, is embedded in an imaginary game of fixed length. Apart from this novel idea, however, such a solution by enumeration is straightforward, but Pascal offered both an independent method based on expectations which is valid for any number of players and, in the *Traité du Triangle Arithmétique*, the solution for two players in terms of the binomial distribution. This, now the standard solution, though derived from the solution by enumeration, was proved by Pascal by induction applied to expectations. The binomial distribution was not given algebraically but by reference to the "arithmetical triangle" of binomial coefficients, whose properties Pascal elaborated in his *Traité* (whence the name Pascal's Triangle).

An original result of Pascal's, given in the *Traité*, is the *combinatorial* proof of the fundamental relation

$$^{n+1}C_{r+1} = {}^{n}C_{r} + {}^{n}C_{r+1},$$

and this work also contains the first formal proof of the long-known binomial theorem for positive integral index.

Late in his *annus mirabilis* of 1654 Pascal suffered an intense religious episode which persuaded him to withdraw from society. Apart from some important work on the cycloid curve, he contributed nothing further of significance to mathematics. He died on 19th August 1662.

R.J. Nelson's book is an addition to the voluminous secondary literature on Pascal which will sit uneasily on a scientist's bookshelf. An examination of Pascal's psychology from the point of view of linguistic philosophy, it is itself written in a language that few will find easy. Thus:

> To be sure, with its emphasis on the nonapodictic notion of figure and its composition in fragments, this most famous work of Pascal [the *Pensées*] does seem to lend itself to a theory of language skeptical of verbal mimesis, to a catachresis suggesting, at least to Marin, that Pascal views all language as aporetic, disjoined from the things, either objects or immutable ideas, that a mimological view of language posits as the referents or signifieds of words or signifiers.

It will be a while before the author's insights into the workings of Pascal's mind influence our understanding of Pascal's science, and that may be a pity, for Part I ("The Adversary") of his book is both interesting and accurate where it touches on scientific matters; but when the Two Cultures are divided by a common language it is inevitable. ☐

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23/30 December 1982

How to get off the MX hook

The US Congress has slapped the Administration in the face by denying funds for MX construction: the problem now, for all concerned, is to find a more manageable missile defence.

The Reagan Administration's defeat in Congress on the MX missile is humiliating but well deserved. Since President Carter's time, it has been understood between the executive and the legislative branches of the United States government that this expensive weapon would not be built until some way had been found of deploying it so as to give both generals and taxpayers some assurance that a substantial proportion of the missiles would survive a first attack from somewhere else. President Carter's favourite solution, shuttling missiles about a dedicated subway system, could have been effective if his Congress had not jibbed at the cost. President Reagan's high-tech solution of the same problem — putting missiles so close together that one attack will neutralize the next — is much more ingenious but even less credible (see *Nature* 2 December, p.389). People skilled at the assessment of the damage done by nuclear weapons say that they understand why "dense pack" should be safe, but acknowledge (as if it were a minor oversight) that they should have given other people more time to understand. What the US Congress has now asked is that if, with the benefit of all the briefings to which it has been privileged in the past few weeks, it cannot understand how dense-packed MX could survive, what reason is there to suppose that a hostile power (*any* hostile power) would be so persuaded of the inviolability of some part of an MX force that it would refrain from a first strike?

The same point bears even more directly on what congressmen's (or women's) constituents think about MX. If a missile-park in Wyoming should fall short of seeming to a potential enemy a viable retaliatory force, why should it seem a good investment of tax dollars to the electors in congressional districts? Congressmen (and women) have learned in the past few years, often against their predispositions, that nothing is as certain to crowd out a community hall, or a town square, than an invitation to protest against MX, or nuclear arms as such or, even, against nuclear things (such as nuclear reactors) in an even broader sense. Latterly, the federal government has been brought face to face with this sombre truth by the knowledge that there is a standing army of a quarter of a million people ready to fill the space around the Washington Monument whenever the call goes out for a protest against something nuclear.

So, in their small ways, congressmen (and women) are already individually paying a price for the MX and what is called its "basing mode". The chances are that even the federal government, a loose synonym for the Reagan Administration, will not be able to survive indefinite rebellion against an unconvincing case for nuclear reinforcement. The danger now is that if the Administration insists that its scheme for the deployment of MX is, for all its faults, the best that it can think of, it will merely widen the gulf between itself and those among its taxpayers who dissent. The Administration has to learn to live with its nuclear opposition.

The military have an important part to play in helping to square this essentially political circle. Many of those who disbelieve in Dense Pack do so because they know in their bones that the technical attributes of the MX missile are an impediment to more sophisticated means of deployment. The ideal, of course, would be that missiles such as MX should be made stochastically invulnerable to destruction from some other missile force by their mobility, and by the opportunities for deception that mobility provides. Putting missiles such as MX in ocean-going submarines may serve well enough for a counter-city force, but the accuracy is not great enough for a missile force intended to knock out other missiles. The simple resolution of this military dilemma, of course, is to acknowledge that MX (which will weigh at least 100 tonnes) is too big to be mobile, and that it would therefore be better to start from somewhere else — say an upgraded version of the submarine-launched Trident, the land-based version of which is known as D-4. Better still, why not go for a much smaller missile that need not weigh more than 10 tonnes or so — mobile enough to be carried on almost any kind of vehicle?

Politically, the US Administration cannot openly embrace such a solution. To embark all over again on the construction of another strategic weapons system would seem like vacillation to the voters and like weakness to potential enemies. Technically, however, there are viable solutions. It would be possible, for example, to build some extra submarines equipped with missiles so as temporarily to keep potential aggressors in their places. That arguments of this kind may have persuaded the Soviet Union, in the mid-1960s, to plan enormously to increase its deployment of strategic missiles is probably, in retrospect, irrelevant. What matters now is merely that the arms race has a curiously irreversible character. If one side builds a new strategic weapons system, the other is likely to follow suit and the result is likely to be a permanent increase of mutual striking power. Building a system like MX is hard enough, but dismantling it is even harder.

Hitherto, the fruits of negotiations on the limitation of strategic arms, such as those that have been taking place at Geneva for almost a year, have been negligible in comparison with what the arms manufacturers have accomplished. Technically, however, there is a way out even from that conundrum: settle for the ratifications of a few simple arms control agreements already negotiated, the threshold test-ban treaty, the agreement to prohibit except under tight controls peaceful nuclear explosions and even Salt II.

The moral, in short, is that if Congress will not give the Administration what it wants, a deterrent that is the opposite of credible in the sense that those who have paid for it do not believe in it, and if (which is not necessarily the case) it is impossible to start again, the Reagan Administration will have to acknowledge that there is no serious alternative to serious negotiations on arms control. As it happens, the Administration has been presented, in the Geneva negotiations on the limitation of intermediate-range weapons in Europe, with an awkward problem on which to brood over the Christmas vacation; the Soviet Union has offered to reduce the number of nuclear warheads based on SS-20 missiles to 300 if only the United States will refrain from putting Pershing II and cruise missiles on their allotted bases in Europe by the end of 1983, and also agree (which is impossible) that the United Kingdom and France should fall in with whatever the superpowers eventually decide. The terms offered are unacceptable not simply because they are unattainable but because they would also be inequitable; they are nevertheless the best prospect at Geneva for a year. The United States should recognize the offer as an opportunity to throw the theatre and strategic weapons negotiations together (where they belong), for winning some arms control agreement below present levels and for escaping from the MX hook.

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Protecting basic research

The US administration plans a generous science budget. It must not look for quick returns.

Curious things are happening at the National Science Foundation, where the ebullient Dr Edward Knapp took over as director a few weeks ago (see *Nature* 11 November, p.100). The good news, or at least the advance reports thereof, is that the federal government's budget for the year beginning next October, to be published when the new Congress has convened in January, will ask the Congress to approve a substantial increase in the foundation's budget by about 18 per cent, well above the rate of inflation (now down to 6 per cent). This development seems to be but a part of the Administration's determination that whatever happens in the next year or two ahead, basic research will not be starved of funds. The principle seems to be that while systematically ridding itself of obligations to carry through further technological demonstration projects — Clinch River is an exception — on the grounds that industry itself should pay for potentially money-making projects, the Administration appears to have acknowledged its own continuing responsibility towards basic research. Other agencies than the foundation, the National Institutes of Health in particular, will now be anxious to find out if the Administration's new-found generosity applies to them.

The other side of the same coin is that the Administration is looking for results — prosperity and all that — but results that it cannot yet clearly define. The argument seems to be that if the Administration has been so good as to agree with academic scientists that basic research is ultimately the wellspring of industrial innovation, and has written its cheques accordingly, it is perfectly within its rights to ask that innovations should flow thick and fast once the cheques are in the mail — and preferably before the next election twenty months or so from now. In the circumstances, it is understandable that Dr Knapp should have been advised to get rid of the three assistant directors whom he inherited (see *Nature* 16 December, p.567); the well known phenomenon that new brooms are rendered ineffectual by the tired servants who use them (to mix a mixed-up metaphor) is in this case complicated by the Administration's need to know who will be responsible for what befalls in the years ahead. The changing of the guard at the foundation is, as of now, politic and not political; Dr Knapp, supported strongly by his previous colleague at Los Alamos, Dr George Keyworth (now the President's adviser on science and technology at the White House), is promising to change the world, perhaps even to break the mould or shift the paradigm; those who will be writing the cheques in the fiscal years ahead want to know who should be blamed if Dr Knapp fails to deliver.

Knapp's prospectus is startling not merely because of the promises it makes on behalf of basic science as the fountain from which innovations spring but because it acknowledges that basic research is educative. Only two years after having offended almost everybody in sight by deleting from the foundation's budget those line-items concerned with the support of science education (in translation, the equivalent of curriculum development in the 1960s mould), the Administration is about to go to Congress saying that the National Science Foundation has a part to play in the education of scientists but this time at a professional level, perhaps by means of partnerships between industry and universities that will be sweetened by modest support from the National Science Foundation. Some of the schemes now being canvassed in Washington are not very different from those tried out in the past decade by the Science and Engineering Council in the United Kingdom. If that is how the budget indeed turns out, the consequence will be that even the next administration (due to be elected in 1984) will not know what to make of Dr Knapp's promises.

What follows is what is called normative or prescriptive advice. Understandably but also rightly, Knapp is impatient that so little (in the way of innovation) has been accomplished by so much expenditure. Among such people, impatience is a virtue. Academic scientists, the foundation's chief pensioners, are almost wilfully indifferent to the needs of industry, their students obstinately persuaded that the academic life is best. A direct attack on these familiar conventions from somebody such as Knapp could help invigorate the system by means of which institutions of higher education train professional scientists. The result, with luck, could be not so much a spate of industrially relevant innovation as a modest cadre of able people. But none of these benefits would show up within what politicians would consider a reasonable time, and certainly not before the next election. More might be accomplished through the National Institutes of Health, which have at least a chance of understanding (not curing) cancer. So Dr Knapp must now keep talking (in public) about other people's opportunities in the hope that he will eventually be recognized as the man who made the National Science Foundation into what it should always have been — a low-budget agency with disproportionately large responsibilities.

Inventing the Wheel

The impending inquiry on nuclear power in Britain promises frustration.

Some years ago (to be precise, in 1972) the Central Electricity Generating Board, the only British public utility empowered to generate electricity for sale to commercial buyers, told a parliamentary committee of its hopes for the future of nuclear power generation. With electricity consumption increasing by seven per cent a year, or doubling every decade, and with the stagnation of coal output, it seemed natural enough that the board should rely for its future sources of energy on nuclear power and oil. (The last strand in the British fuel economy was cut in 1973, when the price of oil was multiplied by between three and four.) In 1972 the generating board was able, a little controversially, to announce that among the nuclear plants it planned to build would ideally be one fashioned on the principles laid down by engineers in the United States, in which the uranium fuel would be enriched to the extent of a few per cent in uranium-235 and in which heat would be carried away to steam generators by means of water under considerable pressure, perhaps 100 times that of the atmosphere. Next year, on 11 January 1983, a public inquiry will be opened in the country town of Sizewell in Suffolk so as to enable the British government to decide whether what is essentially a carbon copy of several hundreds of reactors built elsewhere should be built in Queen Elizabeth's fair isle. With a little luck, construction might begin fifteen years after the first intention was announced.

The inquiry is a public foolishness for which nobody can be excused. The chief culprit, however, is Mr Tony Benn, Minister of Energy in a previous British government who decreed that nothing should be done at Sizewell without a full inquiry. (The present British government weakly assumed Mr Benn's obligation at its election in 1979.) The Central Electricity Generating Board, which has steadfastly refused to confirm the obvious — that the inquiry is a waste of public money and a needless impost on electricity consumers — is almost as much to blame. For a single pressurized-water reactor is not in itself a hazard — and the best way of telling what the hazard would be if there were several such reactors is to build one of them in the first place.

None of this implies that the British government and the public monopolies that it franchises are neglectful of their duty; rather, the opposite is the truth. During the past three years, all those concerned have leaned over backwards to accommodate objectors to the proposed reactor, with the result that quaintly named organizations such as Friends of the Earth have taken to complaining that nobody will pay their expenses to travel in January from their headquarters (usually in London) to Suffolk (less than 100 miles away) to repeat the arguments for which they are already well-known. In much the same spirit might those used to the yoke or litter as a means of transporting heavy objects over uneven ground have petitioned the Roman Senate 2,000 years ago for funds with which to protest at the introduction of wheeled traffic on the newly paved roads.

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Missile spin-off creates activity

MX research an ill-wind brings good

Washington

Research centres around the United States will benefit from President Reagan's determination to press ahead with the MX missile. As long as the fate of the 70-foot long missile and its method of deployment hangs in the balance, lots of money must be spent to keep the teams together and to work on the problems under debate.

At the time of going to press, Congress had effectively deleted $988 million for production of the first five MX missiles, and seemed likely to attach conditions to parts of the $2,500 million MX research and development budget. But most of this amount seemed likely to go forward, as would other parts of the defence budget that related to research, such as funds for Ballistic Missile Defense (BMD) research.

The Ballistic Missile Office of the Air Force pays out this money, most of it to prime contractors, to engineer and test the components of the missile. As with other major weapons systems, the MX's contractors are scattered around the country (often in the districts of important members of Congress).

The Ballistic Missile Office also buys research from other parts of the Air Force to work on the basic problem of what would happen in a Soviet attack and how many MXs would survive. The computers that explore these scenarios belong to the Strategic Air Command, but other offices, such as the Air Force's Office of Studies and Analysis (which judges the "military utility" of total weapons systems), do the analysis.

The "super-hardening" of silos is studied by the Defense Nuclear Agency (DNA), a separate Department of Defense agency whose job is to assess the effects of nuclear explosions. In 1982 DNA reported on a series of tests it had made of blasts over super-hardened silos, which showed they could survive. But the tests were made by combining ordinary explosives to simulate the blast of a 25 megaton nuclear explosion, which makes the conclusion suspect, according to Kosta Tsipis, director of the Massachusetts Institute of Technology programme in Science and Technology for International Security.

Two of the national laboratories, Lawrence Livermore and Los Alamos, are expert in the disturbed environment into which nuclear warheads must fly — and so study problems related to the MX basing mode too.

The Army's BMD programme is also gaining research funds as a result of the need to design a system that could defend the "dense pack" missile field. The BMD programme contracts with private universities such as Stanford, Carnegie-Mellon and the Georgia Institute of Technology.

Outside experts who oppose the plan, such as IBM's Richard Garwin, are few, but powerful. If the dense pack plan is rejected, as other, previous basing modes have been, it may be because the research that underpins it is so scattered.

Deborah Shapley

Dense pack logistics

On 22 November, the President submitted a "dense pack" basing proposal for the new MX missile to Congress to persuade it to release $715 million for research on basing modes in the budget for fiscal year 1983 then being finalized. The plan would put 200 of the highly accurate missiles into a 20-square mile area, at an Air Force base in Wyoming, in silos that would be "super-hardened" so as to withstand a massive pressure of 100,000 pounds per square inch. The MX missiles would be packed 1,800 feet apart, so that incoming Soviet warheads would have to burst near each other and destroy one another through a phenomenon known as fratricide. The Air Force, which runs the MX programme, argued that at least 100 MXs in their silos could survive an all-out Soviet attack; because they would be left to retaliate, the Soviet Union would not attack in the first place.

But many in Congress, including conservatives usually supportive of President Reagan's defence programme, attacked the plan. Bolstered by advice from outside experts, they doubted whether the Air Force could so suddenly have discovered "superhardening", or that fratricide would really occur, or that dense pack would survive exotic methods of attack, such as having the warheads dig up the ground. Moreover, the Air Force admitted that the survivability of dense pack would ultimately depend on building a ballistic missile defence system, something the United States and the Soviet Union virtually renounced by treaty in 1972.

The House voted by 245 to 176 to delete production funds for the missile: the Senate approved the production funds but with a condition that they not be spent until the dense pack plan and the alternatives had been restudied and submitted to the next Congress. The net effect was correction Congress' action was the first serious rebuff to the President's ambitious defence programme. Deborah Shapley

US Congress cops out

Washington

The lame-duck Congress went out with a whimper, not a bang, this week, having failed to act on any of the many science- and technology-related initiatives brought before it this year.

The last hectic hours of the session were taken up with marathon all-night and weekend efforts to agree on a stop-gap bill to keep the government running until September 1983, the end of the fiscal year. The bill that finally emerged from the full Congress, after repeated delaying tactics by Senate opponents of one or another of its provisions, does the following:

- **Clinch River breeder reactor.** $181 million has been provided to keep this controversial project alive. But opponents, who last week thought they had finally delivered Clinch River a death blow when the House of Representatives voted its funds, did win a freeze on construction and a study of the project's usefulness.
- **Regulation of doctors.** The House-Senate compromise knocks out a rider by the American Medical Association that would have barred the federal government from investigating and regulating the trade practices of doctors and other professionals. The amendment had passed the House, but not the Senate.

The science- and technology-related bills left to die include:

- **Nuclear waste disposal.** The United States still has no comprehensive plan for the construction of a permanent repository from commercial nuclear waste or for the interim storage of spent fuel. A legislative initiative in the last Congress, two years ago, was also killed off at the last minute.
- **Immigration reform.** This legislation would have imposed new restrictions on the employment of foreign students after their graduation from US universities. The bill was defeated in the House largely due to opposition from Hispanic groups concerned about its other provisions.
- **Animal rights.** This bill would have required laboratories receiving federal research funds to upgrade their animal facilities and institutional controls on the use of animals in research. The National Institutes of Health may however adopt some of the bill's provisions anyway.
- **Clean Air Act.** A two-year effort to reauthorize the basic air pollution control law and to add new provisions for the abatement of acid rain failed to get out of committee. The present statute remains in effect.

Stephen Budiansky

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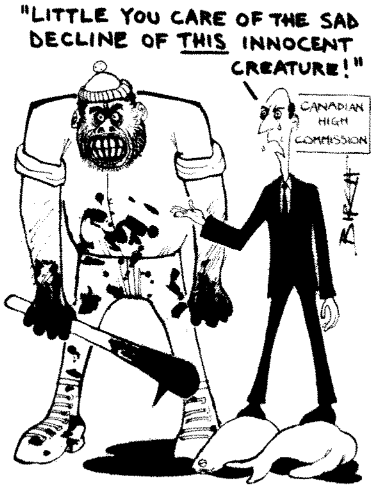
Canada's seals

Who's cooking what books?

The toughly worded resolution approved by the EEC's environment ministers last week to introduce a ban on the import of seal pup skins by its member states comes amid growing concern that the government of Canada (the country most affected by the ban) is making improper use of scientific data to support its opposition.

The relevant scientific questions concern trends and sizes of stocks of harp seals and hooded seals. A recent statement by the Canadian Fisheries and Oceans Minister Pierre de Bané that an October report by an *ad hoc* working group of the International Council for the Exploration of the Sea (ICES) took a "neutral position regarding the hooded seal herd" is described in an EEC memorandum as "utterly wrong and totally without foundation", and catch figures quoted by Mr de Bané are described as "wrong". As to the harp seal, the ICES conclusion — cited in a Canadian government advertisement — that stocks in the north-west Atlantic probably increased between the late 1960s and the late 1970s has since been put down to statistical artefact by several experts.


"LITTLE YOU CARE OF THE SAD DECLINE OF THIS INNOCENT CREATURE!"
CANADIAN HIGH COMMISSION

An attempt three weeks ago by the EEC Environment Council to reach a decision on the proposed ban foundered amid confusion over what legal instrument should be used and what paragraph in the Treaty of Rome should form a basis for the decision. Enthusiasm for the ban ranged from lukewarm (United Kingdom) to fervent (the Netherlands and Italy). The resolution finally approved, unlike a regulation or a directive, is flexible and is not legally enforcible.

Since the abortive meeting of three weeks ago, the Canadian government has placed advertisements in daily newspapers in several European countries claiming that the ICES report, which has been commissioned by Canada and the EEC, "unanimously reported that the harp seal stock of the North West Atlantic has grown significantly during the past decade." According to one scientist whose work formed an integral part of the ICES review, this conclusion "simply isn't there".

The ICES report estimated stocks of harp seals in the late 1960s at 1.2–1.6 million, while in the late 1970s the estimate is 1.5–2.0 million; it says, however, that the possibility that the herd had in fact decreased over this period was "not negligible". It is now thought that the suggestion of an increase is due entirely to a statistical artefact resulting from an attempt to compare estimates based on different methods.

On the question of the much less numerous hooded seal, the advertisements say "there is no indication that this species is in any way endangered". This contrasts sharply with an earlier study commissioned by the EEC from the British Nature Conservancy Council (NCC), which concluded that there was a risk that both species would be endangered by a continuation of the present rates of exploitation, with the hooded seal being most at peril. The ICES report in fact concluded simply that insufficient data are available to provide estimates of hooded seal stocks. Dr Sidney Holt, vice-chairman of the International Union for the Conservation of Nature and Natural Resources, himself a marine mammal specialist, has described the Canadian claims as "gross distortions" of the study's conclusions.

Mr Brian Casey, First Secretary at the Canadian High Commission in London, said of the NCC study that the conclusions were swayed by bias and that anyway it was invalidated by the ICES study. A Canadian government document also describes the NCC study group as "completely dominated by dedicated opponents of the seal hunt" and says NCC is "flagrantly selective" in its use of data. But the two scientists who drafted the NCC report also sat on the ICES working party.

The questions considered by the NCC and ICES reports were substantially different, and there are in fact few actual points of disagreement. The NCC report considered long-term changes in the total stocks of both species, whereas the ICES report was confined to short-term trends in stocks in the north-west Atlantic. The ICES report actually supports NCC's finding that there is a severe shortage of data on hooded seal stocks, which makes reliable estimates of population sizes impossible. It now appears that it ought also to agree with the NCC's conclusion that it is uncertain whether harp seal stocks are increasing or declining. But all this uncertainty may soon be cleared up. The Canadian government is advocating an International Convention on North Atlantic Sealing to provide for expert study and management of all aspects of the seal hunt. **Tim Beardsley & Jasper Becker**

Migrant academics

Mobile Harvard

Washington

Last week, Harvard physicist Sheldon ("Shelly") Glashow, who shared the 1979 Nobel prize, denied widespread rumours that he had been offered a job at Texas A & M university at College Station at an annual salary of $250,000. But Glashow did say he was considering moving there, had visited the university and looked at property. His wife has also visited. Clinton A. Phillips, dean of the faculty at Texas A & M, confirmed that "we are in a negotiation phase" with Glashow, who may spend a year "to see how he likes it".

Glashow would be a catch for the university, which sits on the prairie more than 100 miles from Austin and 90 miles from Houston. Twenty years ago, it had 10,000 students and not a strong reputation. But the state had set aside land in West Texas to help provide for the school, and, like other land in Texas, it had oil under it. So in the 1970s, while the endowments of other, better-known universities shrank, this permanent fund that serves Texas A & M and also the University of Texas at Austin, climbed to $1,700 million —comparable with Harvard's endowment.

Texas A & M has recently lured a new dean to the college of science, John Fackler, a chemist from Case Western Reserve, and provided him with funds for laboratory facilities and staff to continue his research there. Arthur Hansen, formerly president of Purdue University, started as chancellor of Texas A & M last July and was provided with a new home which, Phillips said, cost almost $1 million.

Phillips believes history as well as money is on his side. "In the beginning in this country there was the Ivy League. Then other universities came to be stars: the University of Chicago, Berkeley and Stanford grew in stature. Now our time has come".

Two things have clouded what otherwise seems to be a case of honest academic piracy. The first was that the university's interest in Glashow became known after it hired a new football coach for a comparable figure including television rights and other benefits. The president and chancellor received rises soon after, just to keep their dignity, so to speak. Nonetheless, the new coach had a bad season.

Texas A & M's arch-rival in football is the University of Texas at Austin, which is entitled to two-thirds of the benefits of the permanent fund. In talking to Glashow, Texas A & M may be trying to rival Austin in physics, too. In January 1982, Steven Weinberg, also a well-known Harvard physicist with a Nobel prize, decided to move to the Austin physics department, rejoining his wife, a professor of law, who in 1980 had joined the faculty of the Law School of the University of Texas at Austin. Weinberg's salary is rumoured to

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be $110,000, but he will not confirm this, saying only that his salary is not all that much higher than it was at Harvard.

The university is also giving him an appointment in astronomy and the chance to hire additional faculty to build up the physics department, which he considers excellent in some areas. "It's a very exciting prospect of being able to build a good group", he says. "I don't believe scientists go anywhere for money."

Deborah Shapley

French nuclear power

Cap de la Hague heads on

The French government has finally approved a major extension of the nuclear fuel reprocessing plant at Cap de la Hague, near Cherbourg on the English Channel coast. Meanwhile, a major and partly critical report on La Hague and on reprocessing in general remains unpublished.

The extension will cost some FF20,000 million (£1,786 million) and covers the upgrading of the existing UP2 400 plant to handle 800 tonnes of spent pressurized water reactor fuel and the construction of a completely new plant (UP3) to handle the same amount. The plants would handle French spent fuel and that arising from plants in Japan, West Germany and Belgium with which the French fuel company Cogema already has contracts. Without the extension, the French nuclear power programme would have been compromised. But UP2 400 has been notoriously ineffective — reprocessing only 250 tonnes in its first five years compared with its projected 2,000. After a series of accidents, one of them potentially disastrous (see *Nature* **290**, 538; 1981), the present government set up a scientific commission to investigate the plant.

The report is now complete and should be published in mid-February — but the government has announced that the expansion of La Hague should go ahead before the report is out.

The commission's conclusions, although not public, are believed to be broadly in line with remarks communicated by Professor Castaing, its chairman, to science minister Jean-Pierre Chevènement in April. These were that while Cogema and the Commissariat à l'Energie Atomique now "have the know-how" to build the extension, the technology is only sufficient "for the short and medium term". Castaing told Chevènement that the most satisfactory solution would be "better waste treatment", in particular the isolation of neptunium and americium from the high-level waste stream. The chairman also argued that long-term storage of unreprocessed spent fuel, either wet or dry, was feasible, safe and economical, and must be studied because part of the spent fuel to which France is now committed might remain unreprocessed (if there were problems with the new plant for example). The commission also advocated work on final disposal for glassified waste forms and spent fuel itself. And he added that a fast breeder programme "implies substantial progress in reprocessing". These remarks may have stimulated the government to a preemptive announcement of the La Hague extension.

Robert Walgate

Martlesham in business

An important new semiconductor manufacturing process that was originally developed at British Telecom research laboratories is now being exploited commercially by British Telecom's offshoot, Martlesham Enterprises Ltd, together with Thomas Swan and Co. Ltd.

In January this year British Telecom set up in partnership with other investors to exploit a new process developed by Dr M.M. Faktor and Dr R.H. Moss at British Telecom's laboratories at Martlesham Heath. The process uses a new series of compounds developed in association with Professor D.C. Bradley at Queen Mary College, London, in a technique known as metallo-organic chemical vapour deposition (MOCVD). Existing techniques for MOCVD which used highly toxic and explosive trialkyl gallium and indium compounds had proved extremely hazardous, and had caused several fires and explosions.

The new chemicals will avoid the use of these dangerous compounds and will also increase the range of applications of the method. In particular the process can now be carried out much more effectively with indium phosphide, a material which is likely to find increasing applications in semiconductor devices which emit and transmit infrared light; connected to optical fibres such devices will be important in long-distance optic information links.

Martlesham Enterprises, after a long search, has now awarded manufacturing and selling rights for the new process to Thomas Swan, a specialist chemical manufacturer, which has already secured advance orders for the new chemicals and process equipment from the Massachusetts Institute of Technology.

Tim Beardsley

Soviet science policy

Spread the net

Softly, softly, decentralization and increased local responsibility are emerging as major themes in President Yuri Andropov's economic policy for the Soviet Union. The annual meeting of the Academy of Sciences of the USSR earlier this month suggested that that decentralization should also apply to science. Matching words to good intentions, coverage of the session in the official party newspaper *Pravda* concentrated not on the speech of the academy president, Academician Anatolii P. Aleksandrov, but on that of one of the vice-presidents, Vladimir A. Kotel'nikov, which emphasized the role of the "Republic" academies and the local filials of the All-Union academy.

The occasion coincided with the celebration last week of the 60th anniversary of the founding of the Soviet

INSERM's new shape

French medical researchers are to be subjected to a new form of scientific assessment next year — a mixture of stricter and looser rules than those they face at present.

The new arrangements are part of the long-awaited reform of the Institut National de la Santé et de la Recherche Médicale (INSERM), a reform delayed by arguments over whether directors of research groups should have terms limited to 12 years. That question having been settled — the limit will apply, but not immediately — the way was open for official approval of the full reform. That approval has now been granted, and the details of the reform were published last week.

Among the terms are the new forms of scientific assessment, dear to the heart of INSERM's innovatory director-general, M. Philippe Lazar. Lazar has sought a system which would allow non-conformist — and perhaps superficially mediocre — groups to make their mark. He believes talent, particularly in the undervalued sciences, must be given its opportunity, but that judgements, when made, must be strict and scientific.

The reform meets these two objectives. Groups will be judged infrequently (probably less than annually) but firmly by specialist scientific commissions — which must visit the laboratory concerned. The result will be the definition of a research programme, against which the group will be assessed next time round, with a guaranteed long-term budget.

The mechanism will give groups more freedom and more time from pointless form-filling and fund-hunting, Lazar believes. Robert Walgate

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Union as a federal state. (The exact anniversary falls on 30 December.) Formally, Soviet culture is defined as "national in form and socialist in content", although what this means for science is not always easy to say, particularly since the implementation of the "Kruschchev theses" of 1958 which emphasized Russian as the preeminent language of Soviet scientific communication and thus blurred the individuality of such distinctive developments as the "Ukrainian school" of cybernetics of the early 1960s. Some local institutes have, however, achieved international renown, notably the Paton Institute of Electric Welding (Ukraine), the Institute of Astrophysics and Atmosphere Physics (Estonia), the Byurakan Astrophysics Observatory (Armenia), and the Tien Shan Cosmic Ray Station (Kazakhstan). Their reputations, however, derive from their status as representatives of Soviet science rather than of the republic academies to which they belong.

In creating these academies, from the Ukrainian Academy founded in 1919 to the Moldavian in 1961, the Soviet state was following the federal structure of the Soviet Union which formally says that all 15 union republics are fully autonomous. (Significantly, though, the largest republic of all, the Russian Socialist Federative Soviet Republic (RSFSR), has no academy of its own, being presumably well served by the All-Union academy.)

The propaganda value of separate republic academies is clear. In his jubilee speech, Academician Kotel'nikov reiterated the usual half-truth that the Estonian Academy of Sciences was established in 1945, in the process ignoring the 1938 decree of the then independent Estonian Republic setting up that academy.

Recently, there has been an increasing emphasis on the duty of scientific institutes to service local industry. In the remoter areas of the far-flung RSFSR, former research stations have been upgraded into fully-fledged filials of the Soviet Academy of Sciences.

This theme of regional devolution has been evident since before the death of Leonid Brezhnev and the accession of Yuri Andropov as General Secretary of the Party. The media run-up to the jubilee had begun to stress the links between the republic academies and local production. In particular, the influential weekly *Literaturnaya Gazeta* ran a survey in which the presidents of the 14 republic academies were asked to describe some of the most interesting research carried out by their academies, the main difficulties they encountered and the participation of the academies in the present high-priority food programme. The answers concentrated overwhelmingly on local needs — from the use of lasers for environmental monitoring in Lithuania to soil surveys for new vineyards in Azerbaijan. **Vera Rich**

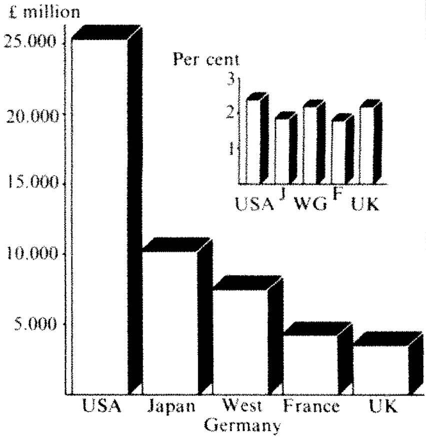
British research policy

Could do much better

Lack of confidence among would-be innovators was cited last week as a major problem underlying Britain's industrial malaise, by Sir Henry Chilver, chairman of the Advisory Council for Applied Research and Development (ACARD). Sir Henry, who was addressing the Parliamentary and Scientific Committee (an unofficial group of MPs, peers and representatives from industry), stressed the importance of science research and development for Britain, and emphasized the need for effective coordination of policies in the many government departments involved with science and technology.

ACARD's terms of reference were broadened in July and the council is now well placed to play a significant role in coordinating research and development efforts in government and industry. ACARD reports directly to the Prime Minister and will also be advising in the recently announced annual reviews of research expenditure by government departments. Its new role was the result of the government's response to the report on "Science and Government" of the House of Lords Select Committee on Science and Technology, which recommended the appointment of a minister at Cabinet level to speak for science and technology.

Sir Henry pointed out that Britain's expenditure on research and development, while comparable with that of its competitors in terms of percentage of gross domestic product, represented only 7 per cent of the total spent in the five major industrial nations (see figure). He therefore argued for more spending on research in private industry and a greater concentration of resources in areas with a potential for later exploitation. He also suggested that more ideas from defence research should be taken up.



Total and relative expenditure on research and development in the five major industrial nations. Relative expenditure is given as a percentage of gross domestic product.

The primary problem, he said, was one of attitudes, and links between universities and industry should be fostered to encourage academics with good ideas to seek support for commercial development.

Few are likely to disagree, but it is far from certain that university researchers and financial institutions will take notice.

Tim Beardsley

India's second taste of Antarctica

Lucknow

The search for a suitable site for a permanent manned research station is the most important task for India's second scientific expedition to Antarctica. Almost exactly a year after the country's first expedition, the 28-member team set sail on 2 December aboard the hired Norwegian vessel *Polar Circle*.

As with the first mission, the landing site is to be near an old campsite at 70° 3′S/40° 7′E, named Dakshin Gangotri (Dakshin — south: Gangotri — the source of the River Ganges in the Himalayas). An automatic weather recording station has been operating there for the past year and the team's first task will be to collect a cassette of weather data.

The present expedition — under the leadership of Dr V. K. Raina, a glaciology expert from the Geological Survey of India — is due to stay on the ice for 60 days, compared with the 10 days of the first mission. Intelsat telex and telephone links have been set up to maintain contact with India's National Institute of Oceanography in Goa and the Department of Ocean Development (DOD) in New Delhi. DOD's link with Antarctica is particularly strong, since the head of the first Antarctic expedition, Dr S. Z. Qasim, is now Secretary of DOD.

With plans to have the permanent base operating by 1985, and ambitions either to build or to buy a research vessel of its own, India's interest in Antarctica is obviously growing. India has not yet signed the Antarctica Treaty. Prime Minister Indira Gandhi said in Parliament recently that her government is examining the case for signing the treaty, and she stressed that the implications for all developing countries are being considered.

India is taking part in discussions with other developing countries about joint efforts to explore and exploit Antarctica. However, offers of help from the Soviet Union, which offered the use of Soviet airstrips and buildings on the ice cap, have been turned down.

Zaka Imam

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Nuclear reactor safety

Super-Sara's fate undecided

Brussels

Community ministers have again failed to reach a "final" decision on the Super-Sara project at the Joint Research Centre at Ispra in Italy to experiment with loss-of-coolant situations in nuclear reactors. Ever since the project was first proposed after the accident at Three Mile Island in the United States, the Community has procrastinated (*Nature* 11 November, p.99), letting the cost of the project soar.

At last week's meeting, the United Kingdom and West Germany again refused to be placated by Commissioner Etienne Davignon's promises to keep the project within the current estimates of 293.9 million European currency units (ECU) (£161.7 million) up to 1988. Vicomte Davignon, the European Commissioner for Energy and Industry, made a passionate appeal, arguing that it would be irrational to cancel the project when attempts should be made to reassure public opinion over nuclear energy. He recalled that the Western economic summit at Versailles agreed to promote international research into nuclear safety and that there was a danger that, if EEC halted its project, the United States would be unwilling to pass on its own research results.

Davignon held that continuing with Super-Sara would not need extra money apart from an extra 13 million ECU for each year between 1984 and 1987. The extra 27.5 million ECU needed for 1982 and 1983 could be found by transferring resources from another part of the budget. And he thought that by reducing funds for other research at the Joint Research Centres, half of the 39 million ECU needed for the three following years could be found comparatively easily.

The United Kingdom and West Germany were not impressed by this financial juggling and asked whether such a large sum of money might not be better employed in some other way.

The upshot is that a panel of three wise men, independent experts from Denmark, France and West Germany, will review the budgetary estimates and assess the value of the Super-Sara project in comparison with that of similar projects elsewhere. Their report, to be presented at the next research council meeting on 8 February, will also take into account the British delegation's suspicion that Super-Sara will absorb too many of EEC's nuclear scientists in an experiment concerned with coping with an emergency rather than on the prevention of disaster.

In the meantime, the European Commission is to think about alternatives for Ispra if in the end the Super-Sara project is cancelled. **Jasper Becker**

Interferon in France

Trial condemned

Paris

Professor Georges Mathé, a well known cancer researcher from the Paul Brousse Hospital at Villejuif, has publicly described as incompetent French doctors involved in preliminary trials with interferon as a possible treatment for cancer. He has criticized the method of administration and the dose and has asked for more information about the circumstances in which four patients involved in the trials died (see *Nature* 11 November, p.97). Mathé has also attacked the establishment where the trials took place and has even asked for the resignation of the members of the scientific council which was set up to monitor the experiments. As a result, the council and IPP (Institut Pasteur Production, the subsidiary of Sanofi–Elf–Aquitaine that produces the interferon) published information about the trials in November. More recently, in a confidential letter addressed to two journalists, they advised wariness of the aggressive tone of Professor Mathé's remarks, which could bring clinical trials of interferon and even clinical trials in general into disrepute.

Two of the four deaths were from myocardial infarction and another has been tentatively attributed to the same diagnosis, but nobody yet knows for certain what caused them. Professor Flamant, president of the monitoring council, says that it is necessary to keep an open mind about the possible role of interferon. But according to Dr Denis Pouillard, clinical physician at the Curie Foundation who participated in the trials, there was a marked similarity between the symptoms and those he had seen ten years earlier in patients given infusions of leukocytes that had been conserved in poor conditions. Dr Pouillard draws attention to the fact that IPP stores its interferon preparation at 4°C whereas -20°C is generally used for leukocyte interferon storage. He also says that since interferon made up only 1 per cent of the protein in the IPP preparation that was injected, it is likely that proteins other than interferon caused the toxicity.

The accidents gave strength to the supporters of gene splicing methods to produce interferon, which give purer and cheaper products. Professor Flamant, Dr Pouillard and Professor Falcoff at the Institut Curie, who is working with gamma-interferon, all advocate that both methods should continue and their products should be compared simultaneously. They would like to see an international body created to compare clinical trials with different kinds of interferon. At the moment, IPP is performing toxicity trials with the suspect interferon and with a tenfold purified derivative. If they show that the purified leukocyte interferon is innocuous, clinical trials will begin again, perhaps in January. **Isabelle Trocheris**

Toxic wastes blow

Brussels

One of the European Economic Community's hardy perennials has reappeared at the latest meeting of the Environment Council, the battle between the methods used on the mainland of Europe and in Britain for calculating the degree to which industrial discharges of toxic wastes should be restricted. Last Christmas, the British persuaded their partners to include in the directives regulating the discharge of toxic substances into water the "quality objective" method as well as the more strict "limit value" system. The latter sets fixed limits on discharges while the former relates the regulations to the use to which the environment concerned is put.

In the first directive, dealing with mercury, the British conceded that whichever system is used, all new plant must use the modern and effective pollution control equipment.

The second directive deals with cadmium discharges, and the French have been pressing for the British to agree that only limit values should be applied to regulations dealing with new plant. The British claim, however, that this breaks the compromise agreement reached last year.

The issue has now been referred back to Coreper, the committee of the member states' permanent representatives, for further discussion, a considerable setback for the Commission. **Jasper Becker**

Jojoba oil

Too late now?

Commercial interest in jojoba oil, once heralded as a substitute for sperm whale oil but since discredited largely because of cost, could be rekindled by a tissue culture technique being developed at Twyford Plant Laboratories in Somerset.

The high price of the oil is largely a reflection of the problems of cultivating jojoba plants. Only female plants produce the oil-yielding nuts and only a small proportion of plants provide a high enough yield of oil to be of commercial value. Attempts to develop the vegetative propagation of high yielding plants have failed because jojoba does not grow well from cuttings.

Now, however, Twyford — part of International Plant Laboratories — and other groups in Israel and the United States have developed a successful approach to propagation by culture of the jojoba's meristem — the growing tip of a shoot or root. The meristem is grown on a series of nutrient media until a seedling develops from it. The seedling is then split into two

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or more parts each of which is grown into a complete seedling. By repeating the process a large population of genetically identical plants is produced. And if the starting meristem came from a plant of high oil yield, the population derived from it by meristem culture should also be high yielders.

Twyford has already shipped clonal populations of jojoba to Arizona for field trials, but admits that the project is still in its infancy, not least because there is a five-year time lag before a plant bears its first crop of nuts.

Even if everything goes according to plan and jojoba oil becomes available at a low price, it is by no means certain that there will be a market for it. According to Highgate and Joab, a major sperm oil refiner now concerned with the development of substitutes, had jojoba oil been available when sperm oil was withdrawn from Britain and the United States, its market would have been assured because it could have replaced sperm whale oil in nearly all of its diverse uses. But industry has since become accustomed to using different oils for different processes and today's market will simply judge whether jojoba oil is better than any of these oils for a particular application. The most likely future for jojoba oil now lies in small specialized fields where no other reasonable alternative exists. **Melanie Kee**

Cancer prize

Washington

The Hammer Prize Foundation has awarded its first $100,000 prize for cancer research to Dr Ronald Levy of Stanford University and George Stevenson of the University of Southampton (in Britain). They were cited for their work on monoclonal antibodies in the treatment of B-cell leukaemia and lymphoma.

The prize, which will be awarded annually for ten years, was established by Armand Hammer, the chairman of Occidental Petroleum. Hammer has offered an additional $1 million for a "cure" for cancer similar to the cure for polio achieved by Dr Jonas Salk. The annual prizes are for the scientists who have made the "greatest contribution towards a cure".

The prize has stirred a certain amount of controversy, both for its origins and for its terms. Some researchers question its focus on treatment, rather than basic research, saying that such an emphasis encourages "premature jabs". And there has been grumbling, too, about Hammer's ties with Occidental, the parent company of Hooker Chemical of Love Canal fame. Hammer's appointment to head the President's Cancer Panel — previously a panel of medical experts — had earlier provoked similar criticisms. Stephen Budiansky

Chemical toxins

USA and UN disagree

Washington

The issue of the use of Soviet toxic weapons in South-East Asia remains disputed. While the US State Department continues its campaign to convince sceptics that Soviet toxin weapons have been used in Afghanistan and South-East Asia, a long-awaited report by a United Nations (UN) group of experts has provided only inconclusive findings.

The most dramatic pieces of evidence now produced by the State Department are two Soviet gas masks obtained in "clandestine operations" in Afghanistan. According to the US Army Chemical Systems Laboratory, which analysed the masks, traces of several trichothecene mycotoxins were found on the surfaces and hose connections of the masks. These fungal toxins are said by the State Department to have been used extensively in Laos and in Kampuchea, where "yellow rain" attacks were first reported.

The State Department has also released analyses of blood, urine and tissue samples from 33 alleged victims of toxin attacks; trichothecenes were found in 16 of the subjects. "The finding of T-2 toxin and HT-2 toxin (a metabolite of T-2 toxin in animals) in the blood, urine and tissue of victims of these attacks provides unequivocal evidence of their use as weapons", the State Department concludes.

But in its report to the UN Secretary-General, the group paints a far more complex picture. Most striking is the utter confusion that the group found in refugees' reports of attacks and claimed illnesses. Two Hmong refugees who said they developed a rash after a chemical attack two weeks earlier were found on examination to have three-month-old fungal infections. Some others who attributed their illnesses to chemical weapons were found to have malaria, or in one case, a gastric ulcer. The group also found discrepancies in reports of numbers of fatalities, the colour of the agent said to have been sprayed and the timing and seriousness of the effects. The group's work had, however, been complicated by the denial of access to Laos and Kampuchea and was limited to interviewing and examining refugees who had crossed into Thailand long after the alleged attacks took place.

The most the UN group was willing to concede was that there is "circumstantial evidence" for the use of "some toxic material" in the areas of Laos inhabited by the Hmong. The group found stories of chemical weapons use in Afghanistan less believable, attributing reports there to incendiary weapons (which would give off irritating smoke as a by-product of combustion) and to a harassing agent such as adamsite. And reports of rapid decomposition of the bodies of chemical attack victims — which have been given prominent attention by the State Department — were "difficult to reconcile with known facts about the effects of chemical warfare agents or other chemical compounds".

Chemical analyses performed for the UN study were likewise inconclusive. Two of the laboratories that analysed blood and leaf samples from Thailand were unable to detect any trichothecenes — even in a control sample spiked with the compounds. A third laboratory reported trichothecenes in all samples submitted, including a blank control.

Furthermore, the possibility of cross-contamination of samples is always present; in addition, the identification of trichothecenes is to some degree a function of the analyst's experience and judgement.

The State Department has tried to avoid argument about its analyses by sending samples to Dr Chester Mirocha, a leading authority on the analysis of trichothecenes. Mirocha's technique is to react samples with trifluoroacetic acid; this produces volatile derivatives of trichothecenes that may then be separated by gas chromatography (GC) and analysed by mass spectrometry. A combination of the GC retention time and the mass-spectrometer peaks can provide "certain" identification, Mirocha says, with a detection limit of less than one part in 10^9. The blood and tissue samples that he analysed contained trichothecenes in the range 10–100 parts in 10^9.

Mirocha points out that even among workers in laboratories with a great deal of experience, quantitative results of trichothecene analyses can vary by as much as 30–60 per cent; in experienced laboratories the variations would not surprisingly be much greater, he says. "Some people have come into this entirely cold. Just because they're chemists doesn't mean they can automatically analyse for trichothecenes", says Mirocha. The UN group used laboratories at the Tokyo University of Science (run by Dr Y. Ueno, who, along with Mirocha, is considered to be one of the world's experts), the University of Veterinary Medicine in Vienna, and the Technical Research Centre in Espoo, Finland (see *Nature* 16 December, p.569).

But reliable as Mirocha's analyses may be, several independent experts question seriously what exactly he has been analysing. The gas masks, for example, were first analysed at the Army's laboratory; Mirocha was sent only a chloroform extract and asked to confirm the presence of T-2. Mirocha says he was also sent a piece of rubber or plastic on which he was unable to detect any of the toxins.

The State Department refuses to say how the masks were obtained, and independent verification of their origin and handling is clearly impossible. Gary Crocker, an intelligence analyst for the State Department, did say that they were obtained in "clandestine" operations specifically planned to obtain evidence of the use of chemical and toxin weapons. One mask was removed from a dead Soviet soldier in Afghanistan in December 1981; the other was obtained in Kabul, Afghanistan, in September 1981. Crocker says "the people who got it were instructed on how to handle it", and were provided with a similar sample container that would prevent contamination. "Things were handled properly all down the line".

Dr H. Bruno Schiefer, who conducted a study of toxin weapons use in South-East Asia earlier this year for the Canadian government (a study that found effects that could not be explained by "naturally occurring phenomena"), says "It doesn't really matter who" provides samples — the results "still don't stand up in court". Schiefer says "we would need some sort of verification" by scientists who could go into the affected areas, collect samples and bring them back for analysis.

Stronger criticism comes from Arthur Westing of Hampshire College. "The US has a long history of doctoring evidence", he says. "I don't know if the State Department is lying, but they haven't come close to proving their case." Westing says that "the most elementary mistake they made" was not to get blood and urine samples from a control population outside the area where toxins were allegedly sprayed. The parts-per-billion levels of trichothecenes found in the medical specimens from alleged victims may, he says, reflect natural exposure to the toxins: "There's no question that the *Fusarium* fungi that produce these toxins do occur in that region. The *Fusarium* fungus responsible for T-2, for instance, was described in the 1930s as occurring in Vietnam, according to Westing. And Westing points out that aflatoxin, well known to contaminate food, was found in one of the alleged victims at a concentration comparable with that of T-2.

Mirocha counters that his experiments with cows show that relatively large amounts of T-2 — hundreds of milligrammes — have to be ingested before it shows up in the blood at even the 10–100 parts in 10^9 level. "To say it's present endemically in the food is absurd", he says.

The State Department, for its part, seems less concerned with technical details than with the overall pattern of evidence. "I could take any piece of evidence and give you an alternative hypothesis", Crocker says, but the "strength of the case" lies in the agreement among reports from refugees and defectors, intelligence information and laboratory analyses. That argument is not likely to convince many sceptics; definitive proof may come only when an independent group of experts is allowed into Laos and Kampuchea.

Stephen Budiansky

Tasmanian environment

Dam threatens wilderness

Canberra

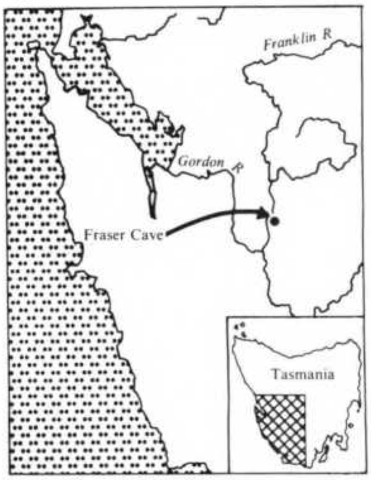
An area rich in archaeological sites from the last ice age will disappear beneath a flood of water as part of a hydro-electricity project unless Australia's national government overcomes its reluctance to interfere with a state decision. Last week Unesco's World Heritage Committee, meeting in Paris, placed the Western Tasmania Wilderness National Park on its register, thus increasing the pressure on the Tasmanian state government to reconsider its plan to build a dam on the Gordon River, just below its confluence with the Franklin River. The dam would inundate 37 kilometres of the Gordon, 37 kilometres of the Franklin and part of all the tributaries of the Gordon including all the recently discovered archaeological sites.

The national government is now left in a curious position. In November 1981 it backed the then Labour state government in applying to put the national park on the heritage list. When a newly elected Tasmanian government later decided to allow the Tasmanian Hydro-Electric Commission to build the dam the national government came under pressure to stop the state scheme. Finally, on 8 December, the federal cabinet decided not to interfere.

Professor John Mulvaney of Australian National University resigned from the council of the Museum of Australia in protest at the decision, arguing that the threatened area qualified for the heritage list under the United Nations convention's criteria for both cultural and natural properties — as well as having archaeological value the area contains an important natural habitat for huon pine trees, some as much as 2,000 years old.

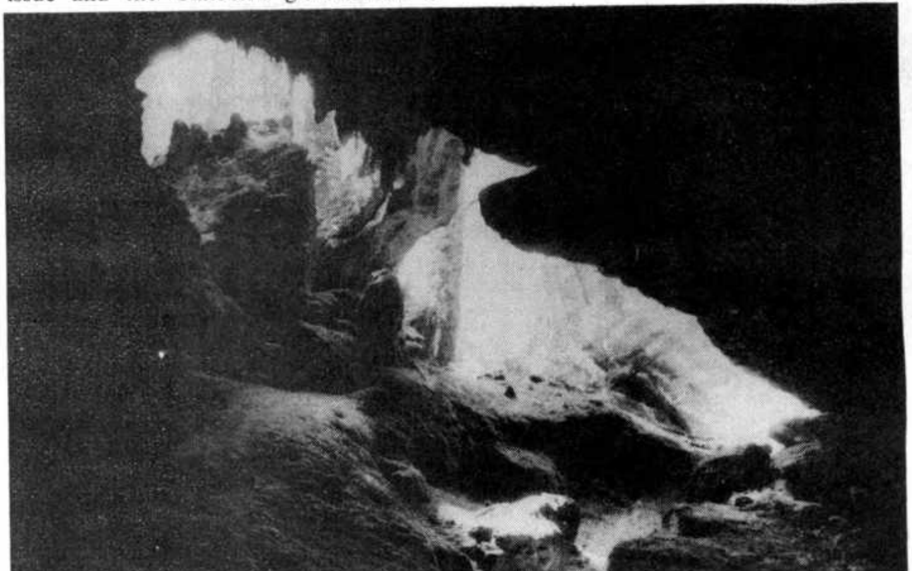
The relationship between national and state governments in Australia is a sensitive issue and the Canberra government is reluctant to oppose decisions made at state level. In this case the hydro-electric scheme is seen as a way of attracting industry to an area with the highest unemployment rate in Australia — 12 per cent — so it would be a major step for politicians to oppose it.

The farthest the government has so far gone in defence of the national park is a statement from Mr Thomas McVeigh, minister for home affairs and conservation, to the House of Representatives, saying the government "intends to enter discussions with the Tasmanian government with a view to minimizing the damage that the dam might cause . . . it will look towards an agreed management plan for the total area".



The argument that seems to have clinched the decision at last week's Paris meeting is that placing the national park on the heritage list will increase its chances of being preserved. Even so, the Tasmanian government is claiming that the listing gives the dam "the green light to go ahead" — a deliberate misinterpretation it seems.

Vimala Sarma



The entrance to Fraser Cave — due to be submerged if Tasmania's dam scheme goes ahead. In January last year stone tools and human remains dating from the last ice age were found here. The findings are reported in detail in the next issue of *Nature* (6 January 1983, p.28).

0028-0836/82/510679-01$01.00

CORRESPONDENCE

Pole imprisoned

An open letter to the President of the Polish Academy of Science

Paris, 24 November 1982

SIR — We would like to raise the question of the fate of one of our Polish colleagues, Antoni Stawikowski, a matter which is of great concern to us.

Antoni Stawikowski is a research scientist at the Nicolas Copernicus Centre for Astronomy at Torun. He occupies an important position in the Polish astronomical community, having recently been appointed vice-president of the Polish Astronomy Society. Following his election in 1981 to the position of regional manager of the independent trade union Solidarity he asked for his responsibilities at the research institute to be temporarily suspended so that he could devote his attentions to his union duties.

He was interned on the night of 12/13 December 1981 following the declaration of a state of war in Poland. After a period of detention at Potulice prison he was transferred to the Strzebielinek internment camp. He was freed in August 1982 and returned to his research at the Nicolas Copernicus Centre for Astronomy at Torun.

Now, we have just learned that he appears to have been imprisoned again. We would be very grateful if you would outline the exact motives behind this new imprisonment and specify precisely the nature of the proceedings being brought against our colleague Antoni Stawikowski. We would also like to know his conditions of internment.

Whilst waiting for what we hope will be your rapid reply, we would like you to accept, Mr President, our respectful good wishes.

E. SCHATZMAN
(President)

On behalf of the Council of the Société Française des Spécialistes d'Astronomie, Paris, France

Chinese conundrum

SIR — M.C. McKenna incorrectly implies (*Nature* 18 November, p.212) that, in transcribed Chinese, disyllabic names are all given names and monosyllabic ones surnames. The reverse is true in the name provided for me by Chinese friends: Situ Li. Note also that in *pinyin* romanization there is no hyphen in disyllabic names. Incidentally Chinese names are not the only ones to give problems: a German correspondent once addressed me as "Herr Professor Doktor St. Philip".

PHILIP J. STEWART

Commonwealth Forestry Institute, Oxford, UK

Napoleon's health

SIR — In *Nature* of 14 October there were two contributions that bear relevance upon my own theory of criminal poisoning of Napoleon at St Helena. Lewin *et al.*[1] found no arsenic in a lock of hair obtained by Captain Poppleton, one of the British officers at St Helena. The sample did contain a moderately increased level of antimony, however.

Napoleon's health improved considerably during the first 9 months of his captivity. The length of hair that grew on Napoleon's head during this period can be estimated at 9-10 cm. The lock of hair obtained by Poppleton may well have been from this period.

It is more difficult to explain why the analyses showed an increased level of antimony. It is true that antimony is a component of tartar emetic, which was used as a medication, but it is also known that Napoleon stubbornly refused to accept medicines. As far as we know, Napoleon had no symptoms of antimony poisoning before March 1820. If the results of the analyses are correct, one is forced to question the authenticity of the sample.

One statement by Lewin *et al.* needs correcting. My theory of arsenic poisoning[2-4] was not based mainly on the high contents of arsenic found in hairs from Napoleon, but mainly on written evidence (diaries, letters, postmortem reports). The results of analyses of hairs must be seen as a confirmation.

Jones and Ledingham[5] found arsenic in wallpaper from the drawing room of Longwood. The authors mention that mould on damp wallpaper can metabolize arsenic compounds to volatile arsenic trimethyl. Leaving aside the question of how damp Napoleon's wallpaper was, the fact remains that the clinical picture is not one of continuous low-level poisoning. Napoleon repeatedly suffered from very severe attacks of illness; these were separated by periods of recovery. Analyses of hairs confirm that Napoleon repeatedly consumed relatively large amounts of arsenic.

For about 15 months, from July 1819 to September 1820, Napoleon enjoyed a relatively good health. During this period he could again go for walks, take part in gardening, and go horse riding. Napoleon's disease proceeded independently of any constantly active cause.

Furthermore, Napoleon did not live alone in his house. He was surrounded by an impressive staff of Frenchmen. The diaries do not report any symptoms of chronic arsenic poisoning among these. All this indicates that the wallpaper was not a major source of arsenic in Napoleon's case.

It is a common misunderstanding that Napoleon was killed by arsenic poisoning. The cause of his death was a combination of a liquid refreshment containing bitter almonds and a large dose of calomel given 48 hours before his death. Napoleon soon fell into deep unconsciousness (the effect of mercuric cyanide). The internal surfaces of his stomach became corroded (the effect of corrosive sublimate). The main purpose of the slow arsenic poisoning was to give observers the impression that Napoleon had some chronic disease, thus making his death appear more natural. Further details about the death-blow can be found in ref. 3.

STEN FORSHUFVUD

Göteborg, Sweden

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Napoleon's arsenic

SIR — I wish to make the following comments on the article by Lewin *et al.* in *Nature* of 14 October (**299**, 627-628; 1982) which reported very much lower levels of arsenic in Napoleon's hair than we found (*Nature* **194**, 725; 1962).

The separation we used was a radiochemical one and the interference referred to by the authors as a possible explanation of our detection of high levels of arsenic is below the 1 per cent level. This is easily demonstrated by using the method.

Gamma-spectrometry as used by Lewin *et al.* is a poor method for arsenic analysis. Though for other investigations we use similar equipment (now standard) to that mentioned by the authors we still use the radiochemical separation for trace analysis. We have applied the technique to the analysis of several thousand of standards and samples since the investigation of Napoleon's hair.

HAMILTON SMITH

Department of Forensic Medicine and Science, University of Glasgow, Glasgow, UK

Reprinting history

SIR — Reprints (also known as extracts, offprints) of articles from scientific journals have been used as a form of scientific information exchange for over two hundred years. Scientists have, since the early nineteenth century, acquired and collected them for their personal professional libraries. One well-known reprint collection is that of Wolfgang Ernst Pauli at the European Organization for Nuclear Research, Geneva (a list of it was published in 1969).

In connection with a study of the history of the reprint, I would appreciate hearing from scientists about the earliest reprints they have seen, what nineteenth century collections they may know of which may have survived, and the importance they attach to such collections.

ELLEN B. WELLS

Smithsonian Institution Libraries, Washington DC, USA

Oncogene activation

SIR — The article by Reddy *et al.* (*Nature* **300**, 149; 1982) describes a relatively minor change in DNA structure which results in the activation of the T24 human bladder cancer oncogene. The change is a point mutation which according to the authors is a "mutation of guanosine into thymidine". Obviously, the intent of the authors was to describe a deoxyguanosine to thymidine transition, or better yet a mutation of guanine into thymine. Their error may seem trivial and perhaps amusing, were it not for the fact that such minor differences can have apparently significant consequences on the properties of genes, as shown by the work described in the paper. It is disconcerting that such a fundamental mistake was published in such an important article.

LASZLO BERES

Hull, Massachusetts, USA

NEWS AND VIEWS

Disputes about the Earth's interior

Sir Harold Jeffreys and Dr R. A. Lyttleton are again at loggerheads. Both are mistaken to pretend that plate tectonics does not exist. Lyttleton is right to give another airing to Ramsey's theory.

The most conspicuous of those who reject the theory of plate tectonics is Sir Harold Jeffreys, now 91, who founded the school of geophysics at the University of Cambridge and who, with successive editions of his monumental book *The Earth* (the sixth was published in 1976), has spread his influence far and wide. Jeffreys is not so much an iconoclast as the opposite; far from being prepared to break the icons of the past, he would keep them bright and shining, as can be gathered from his insistence that the successive periods of mountain-building recognized by classical geologists must be explained by a shortening of the Earth's crust in turn caused by the contraction of the body of the Earth in the past 4,500 million years.

As it happens, Cambridge is also the base (some would say refuge) for another distinguished supporter of that view, Dr R.A. Lyttleton, who may nevertheless be better known for his contributions to celestial dynamics and to the theory of comets. So has Cambridge (where Vine and Matthews first demonstrated that the sea floor really spreads (see *Nature* **199**, 947; 1963)) become a place in which ideologically motivated men conspire to undermine the theory of plate tectonics? Not a bit of it. Far from making common cause, Lyttleton and Jeffreys have become persistent critics of each other's work. Their discontents, with plate tectonics but also with each other, are nicely illustrated by their latest contributions to the scientific literature.

First, Jeffreys. This month's issue of the *Geophysical Journal of the Royal Astronomical Society* (**71**, 555-566; 1982) includes Jeffreys's article "Tidal friction; the core; mountain and continent formation" in which he concedes an important point that Lyttleton has been urging on him for the past five years — that in attempting to relate the observed deceleration of the Earth's rotation to the influence of tidal forces, it is essential to allow for the possibility that the moment of inertia of the Earth may be changing. This important issue has its origins in Newton's theory of the motion of the Moon and in Halley's observation, in 1695, that compared with the predictions of that first serious essay in celestial mechanics, the Moon appeared to be decelerating in its orbit about the Earth. By the mid-nineteenth century, the reality of the deceleration (and that of the Sun and planets) had been firmly established and ascribed to the consequences of tidal friction. There are two effects — the Earth's rotational velocity is decreased, but (because angular momentum is conserved) the Moon retreats from the Earth (by about 3.5 cm a year) into an orbit in which the proper motion is decreased.

As it turned out, the first accurate estimates of the quantities concerned appeared only in the 1930s, when Spencer Jones (then the Astronomer Royal of England) was able to distinguish the secular deceleration of the Moon from the apparently random fluctuations of the Earth's rotational speed — a task then complicated by the use of the Earth's rotation as the standard of time. He estimated the secular acceleration of the Moon as -22.4 4.0 seconds of arc per century per century, well within a standard deviation of the values more recently obtained by laser ranging, from the longitudes on the surface of the Earth at which ancient eclipses were observed and by combining accumulated accurate observations of the planets. Jeffreys (like others) prefers a larger value, agrees with Lyttleton that the data do reveal changes of the moment of inertia of the Earth but considers that these may amount to a few parts in 100 million over the course of a decade. The explanation of recent fluctuations, Jeffreys says, is to be found in climatic change and the consequent redistribution of ice and water over the surface of the Earth. But attempts to pin down the long-term non-tidal contribution to the acceleration of the Earth's rotation should wait on more accurate observations even though the crustal shortening of at least 200 km needed on Jeffreys's view to account for mountain-building must have gone some way to offset the deceleration caused by tidal forces.

Lyttleton has no such inhibitions. His latest book, *The Earth and its Mountains* (Wiley, 1982), published in November, is a splendid *tour de force* intended to bring together his own interpretation of the secular acceleration of the Moon with a theory of the Earth's interior first put forward thirty years ago by the late W.H. Ramsey. As a contribution to the literature, Lyttleton's book has the virtue of being neither a didactic work nor an exercise in popularization but an argument addressed to people in all disciplines able to stomach some elementary algebra. On the basis of the ancient eclipse data, he concludes that the intrinsic acceleration of the Earth's angular velocity due to its decreasing moment of inertia is between a fifth and a quarter of the deceleration expected from the tidal forces of the Sun and Moon. (Lyttleton acknowledges R.H. Dicke's estimate in 1966 of an intrinsic acceleration only a little less.)

Lyttleton bravely and persuasively puts his faith in Ramsey's theory — the view that the molten core of the Earth differs from the mantle above it not in chemical composition but because of a phase transition from solid insulating rock to material of essentially the same composition in a liquid and electrically conducting phase. The theory originally advanced as a means of accounting for the average densities of the terrestrial planets (the Moon included) has the great advantage of consistency and simplicity. One of its particular virtues was to avoid the implausible assumption, current in the 1940s and 1950s, that the molten core consists solely of an alloy of iron and nickel. Shockingly, the papers presented at the Royal Society on the Earth's core in January this year include not a single reference to Ramsey's work (see *Phil. Trans. R. Soc.* **306**, 1-289; 1982) even though most recent attempts to account for the composition of the core involve attempts to "lighten" it with the admixture of elements such as oxygen, sulphur and silicon. The chief objection, that shock-wave and high pressure measurements with materials such as olivine have failed to demonstrate the required phase transition, may be irrelevant. Lyttleton is entirely right to carry a torch for Ramsey's theory, which is in that category of theories too neat not to be true. Needless to say, the steady growth of the Ramsey–Lyttleton core neatly accounts for the observed decrease of the Earth's moment of inertia, the contraction of the Earth's diameter in past aeons and thus for mountain-building.

Where does plate tectonics fit in? Lyttleton does not mention this other way of building mountains. Jeffreys refers with regret to the defection of Sir Arthur Holmes to "Wegener's theory" and says that attempts to account for the subduction of oceanic plates must be likened to "cutting butter with a knife also made of butter". Time will probably show that all the participants in this many-cornered quarrel are correct, or partially so. Lyttleton has scored an important point off Jeffreys, but Jeffreys is probably right to insist that the uncertainties about the motion of the Moon and composition of the Earth's core are still too great for a simple resolution of this long-standing dispute. Both of them should also admit, however, that the case for plate tectonics is too strong to be ignored, and both should admit that Ramsey's theory could yet turn out to be more interesting than they acknowledge. In its original version, the theory supposed that the solid rock of the mantle and the molten material of the core were identical in composition. But every chemist knows that multi-component systems in equilibrium with each other may differ in composition.

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Onco Gen

from Peter Newmark

A pair of papers in this issue implicate two human genes, both of which have some pretensions to be called oncogenes, in the causation of specific cancers that are associated with chromosomal rearrangements.

The more advanced study is that of de Klein and his colleagues on page 765. They show that c-*abl*, the human gene homologous to the oncogene of Abelson murine leukaemia virus, is translocated from chromosome 9 to chromosome 22 in the leukocytes of three individuals with chronic myelocytic leukaemia (CML). The implication is that c-*abl* is activated as a result of its translocation and that the activation is associated with the disease.

The translocation between chromosomes 9 and 22 is found in over 90 per cent of cases of CML. One result is the so-called Philadelphia chromosome — chromosome 22 without its long arm. Another is the addition to chromosome 9 of the deleted section of chromosome 22. It has also been generally assumed that there is a reverse transfer of material. De Klein and colleagues prove that point by establishing that c-*abl* has shifted from chromosome 9 to the Philadelphia chromosome of CML leukocytes.

Highly suggestive, though this is, of an association between the c-*abl* translocation and the disease, it is only a beginning. Evidence will be needed that c-*abl* is activated by its shift to chromosome 22 perhaps by coming within the active domain of the immunoglobulin λ light-chain genes on that chromosome; and there is the unproven assumption that activation can lead to transformation. The viral gene certainly seems involved in transformation. It produces a tyrosine-specific kinase in Abelson murine leukaemia virus-induced lymphoid neoplasms of mice (although an unrelated transforming gene from such neoplasms transforms NIH3T3 cells in the standard assay for oncogenes — see *Nature* **300**, 659) but there is no evidence yet for a similar product in CML leukocytes.

Note also that c-*sis*, the human homologue of another viral transforming gene — that of the simian sarcoma virus — is shifted the other way in CML: from chromosome 22 to chromosome 9.

An even more tentative link between a human homologue of a viral transforming gene and a tumour with an associated cytogenetic abnormality is reported on page 773. The virus is Harvey murine sarcoma virus; the human gene has already been implicated in human bladder carcinoma (see p.762). McBride *et al.* now assign the normal allele of the gene (c-*bas*) to human chromosome 11 and point out that it is deleted in Wilm's tumour.

Caged ATP set free in muscle

from Peter D. Chantler

THE most noble aim of the biochemist, often discussed when inebriate, seldom when sober, is to relate the *in vitro* to the *in vivo*. This is never more true than in the field of muscle contraction where ordered arrays of aligned fibrils and interdigitating filaments have bolstered confidence that this elusive goal could be just around the corner, achievable by a plethora of physical techniques. And X-ray diffraction has come remarkably close. The recent time-resolved studies on isometrically contracting muscle[1,2] clearly indicate the power of this technique; evidence for a change in crossbridge orientation has been obtained on a millisecond time scale. Despite such advances, the democratic world of atomic masses will inevitably obfuscate a complete correlation of the *in vitro* with the *in vivo*. Other techniques are needed. One approach, due to Goldman, Hibberd, McCray and Trentham (see this issue of *Nature*, p.701), makes use of a novel molecule — caged ATP.

Caged ATP was first introduced by Kaplan and co-workers in 1978 (see ref. 3) who used it to study the (Na$^+$ + K$^+$)-ATPase in resealed erythrocyte ghosts. It is a chemically inert form of ATP, being a nitrophenyl derivative conjugated as the γ-phosphate ester of ATP. Photolysis at the suitable wavelength cleaves the molecule so as to yield ATP and 2-nitrosoacetophenone (see the figure and refs 3,4). The use of a frequency-doubled ruby laser at a wavelength remote from most protein absorption bands (347 nm) demonstrated a linear relationship between the degree of photolysis and the energy output of the laser[4]. Thus it became possible to design an experiment in which a known amount of ATP could be generated in a muscle fibre simply by controlling the total concentration of caged ATP and the energy of the laser pulse. Furthermore, caged ATP does not itself act as either substrate or inhibitor for the (Na$^+$ + K$^+$)ATPase[3] or for actomyosin[4] — a vital attribute if one wishes to monitor events on a millisecond time scale.

The first experiments performed by Goldman and colleagues (see p.701) were designed to determine the rate of detachment of crossbridges in rabbit skeletal muscle fibres. Single skinned rigor fibres were immersed in a bathing solution (no calcium) containing caged ATP which diffused into the fibre; no change in rigor tension resulted. At zero time, a pulse of 347 nm laser light (30 ns) was applied to liberate a predetermined burst of ATP, resulting in relaxation of the muscle fibre. Both tension and muscle stiffness were monitored. By varying the ATP concentration, the second-order rate constant for detachment could be measured and was found to be 2×10^5 mol^{-1}s^{-1} at physiological ionic strength and neutral *p*H, identical to that obtained for actomyosin dissociation[5] (0.5 M KCl) and only a factor of five lower than that for acto-HMM[6] (0.05 M KCl). The agreement suggests that the detachment rate constant is not substantially altered by the structural constraints of the filament lattice.

The effect of an imposed change in length, one second before the laser flash and consequent ATP release, was also investigated. If the rest-length rigor fibre (no calcium) was stretched 30μm before ATP release, the initial tension (similar to the force of an isometric contraction) decayed rapidly on photolysis with a half time of less than 10 ms. Alternatively, if the rigor fibre (no calcium) was released by 30μm before the laser pulse, the lower initial tension either remained constant or even increased before the final relaxation. The final phase of all relaxation transients superimpose. The rise in tension, following ATP generation after a short release step, is not an artefact of the system. Goldman and co-workers present a convincing argument that this effect is a physiological consequence of the type of thin-filament cooperativity first proposed by Bremel and Weber[7]. As noted above, detachment rates determined in the absence of calcium are fast; reattachment rates, determined by performing similar release experiments in the presence of calcium, are also fast and in agreement with X-ray results[2]. If, therefore, the laser-induced production of ATP in the fibre results in detachment of a large fraction of attached crossbridges and yet still leaves a proportion of rigor bonds (myosin heads without bound nucleotide), then even in the absence of calcium, force-generating crossbridges will re-attach because the rigor bonds push tropomyosin

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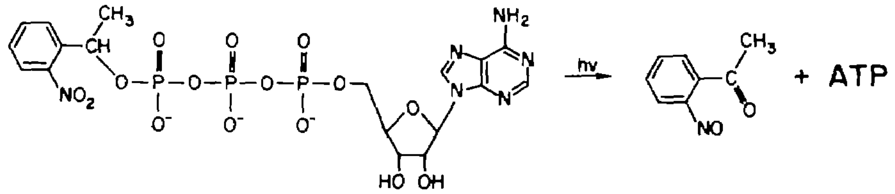

Cleavage of caged ATP by light.

away from its blocking position[7]. The result will be a transient increase in tension before relaxation. Measurements of stiffness indicate a gradual decline in the total number of attached crossbridges throughout these events, indicating net detachment. As a *pièce de résistance* Goldman and collaborators model these release experiments using a three-step cycle involving active, rigor and detached bridges. Model and experiment are in good agreement.

Another approach of particular note, due to Thomas and co-workers, also relates the *in vitro* situation to the *in vivo* one using electron paramagnetic resonance (EPR) spectroscopy[8-11]. Spin labels, selectively and rigidly attached to myosin heads in glycerinated rabbit psoas fibres, have allowed the acquisition of data concerning head orientation[10] and submillisecond motion[9] during rigor and relaxation. The latest paper in this series (Cooke, Crowder and Thomas, this issue of *Nature* p.776) examines the orientation of spin-labelled heads during an isometric contraction. Labelled rigor muscle fibres show a distinctive EPR spectrum indicative of a highly oriented population of heads; a very different spectrum is obtained from the same fibres during relaxation, indicative of isotopic orientation[10]. When Cooke and colleagues add calcium to relaxed fibres causing an isometric contraction, the resulting EPR spectrum can be broken down into a summation of spectra from relaxed and rigor muscle fibres in the ratio of 4:1. No other orientations are seen; controls were performed to ensure that all heads were saturated with nucleotide.

Now, the paper of Goldman *et al.* (p.701) puts an upper limit of one per cent on the proportion of time a crossbridge spends as a true rigor bond during an isometric contraction. Hence the observation of 20 per cent spin-labelled heads having a highly ordered rigor-like EPR spectrum during an isometric contraction (Cooke *et al.* p.776) leads one to an ineluctable conclusion: the domain probed by the spin label on the myosin head does not change its orientation during the powerstroke. This conclusion is consistent with the observation that an applied stress imposed on rigor muscle fibres also does not alter the orientation of the domain probed by the spin label[11]. Nevertheless, although part of the myosin head may well be immobile during the power stroke, the demonstration of work production by actomyosin machines using only soluble proteolytic subfragments of myosin (myosin heads)[12] restricts the conformational changes necessary for force generation to other domains on the head itself, rather than the myosin tail. □

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Transcellular cross-talk between epithelial cell membranes

from Jared M. Diamond

OUR bodies are catacombs of spaces connected to the outside world and filled with fluid. The spaces include the lumina of the intestine, kidney tubules, and salivary and sweat ducts. Separating these spaces from the bloodstream are cell sheets called epithelia, which secrete or absorb fluids by pumping ions from the blood into the spaces or vice versa. An enormous boost to epithelial research came when the Danish physiologists Ussing and Zerahn[1] devised a new method of measuring the pumping of ions in 1951. Seven years later Koefoed-Johnsen and Ussing provided a second leap forward with a new model to explain the pumping[2]. The model has evolved considerably in subsequent years, and this evolution is in turn relevant to the theories of scientific models proposed by historians of science. Several papers published in recent months report further significant advances[3-5].

The Koefoed-Johnsen and Ussing (KJU) model sought to explain sodium absorption, a dominant transport mechanism in many epithelia. In the 1950s, students of nerve, muscle and erythrocytes had discovered how these cells regulate intracellular cation content through three membrane processes: passive permeation of Na^+, passive permeation of K^+, and active ATP-driven extrusion of Na^+ coupled to uptake of K^+. The KJU model was couched in terms of these same three processes and postulated their restriction to one or other face of the sheet: passive Na^+ entry to the cell membrane facing the lumen, and passive K^+ exit and active Na^+-K^+ exchange to the cell membrane facing the blood (Fig.1*a*). Part of the model's appeal was that it postulated no new processes beyond those already known to regulate ion movement between cell interiors and the blood. The limitations of these postulates were also the major focus of the model's further evolution.

Epithelia are not merely polarized cells arranged in a sheet. Each cell in the sheet is joined to its neighbours by junctions, which in principle offer a path across the epithelium that by-passes the cells. The KJU model, and epithelial research of the 1950s generally, ignored the possible importance of this path. In the late 1960s and early 1970s, when new techniques became available, it turned out that the alternative route was not, indeed, important for one class of epithelia, now termed tight epithelia. In such tissues, exemplified by frog skin, mammalian and amphibian urinary bladders, and stomach, ions cannot pass through the junctions and must traverse the epithelium via the cells. However, in 'leaky' epithelia, exemplified by renal proximal tubule, small intestine and gallbladder, the junctions are the major route for passive transepithelial movement of ions. Tight and leaky epithelia have subsequently been found to differ in the way in which Na^+ enters the epithelial cells across the lumen-facing (apical) membrane. In tight epithelia, as well as in the medium-tight large intestine and renal distal tubule, Na^+ enters by a highly specific mechanism that is inhibited by very low concentrations of amiloride and that recent measurements of electrical noise have shown to be an ion channel[5,9]. In contrast, in leaky epithelia Na^+ entry is coupled to the entry of a partner (a non-electrolyte such as sugar or amino acid, or Cl^-), or perhaps to H^+ exit in parallel with Cl^-/HCO_3^- exchange. This process is believed, but not firmly proved, to involve a carrier[10,11].

A recent modification of the model came from the discovery that the pump at the blood-facing (basolateral) membrane does not swap 1 Na^+ for 1 K^+, as originally assumed, but rather 3 Na^+ for 2 K^+, as is also true for nonepithelial cells[5,12-14]. More major changes have, however, focused on how ion movements at the apical and basolateral membranes regulate each other[15]. Apical Na^+ entry is greatly stimulated by physiological agents such as luminal nutrients, aldosterone and antidiuretic hormone. What prevents the rate of apical Na^+ entry from exceeding that of Na^+ extrusion by the basolateral pump, leading to osmotic lysis? Increased basolateral Na^+ extrusion is coupled to basolateral K^+ uptake: how is the cell similarly protected against osmotic lysis through increased K^+ content? Other physiological agents, such as CO_2 and insulin, stimulate basolateral Na^+ extrusion: what then signals the apical membrane to become more permeable to Na^+, lest apical Na^+ entry become rate-limiting? Do conditions that stimulate

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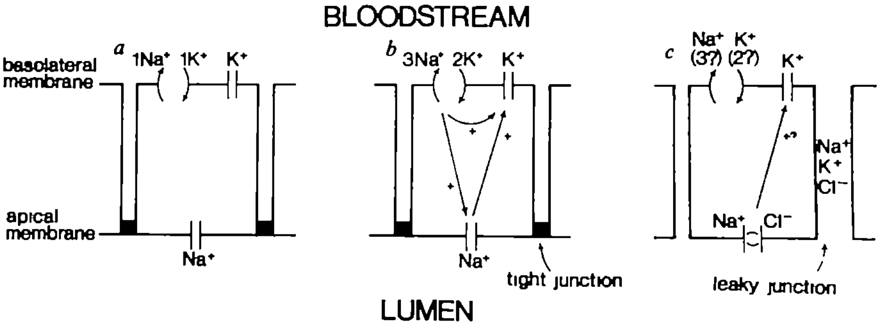
apical Na$^+$ entry then stimulate basolateral Na$^+$ extrusion through a rise in cell Na$^+$, or is there a different signal? In retrospect, our understanding of epithelial transport in 1958 resembled an economic study of the banking system that considered savings inflow and loan outflow, without having begun to ask how inflow and outflow regulated each other by determining savings and loan interest rates.

The first indication of cross-talk between apical and basolateral membranes came in 1961, when MacRobbie and Ussing[16] used cell swelling as an indirect measure of ion entry in frog skin. They found that inhibiting the basolateral pump reduces the ionic permeability of both the basolateral and apical membranes. The effect on apical Na$^+$ permeability has now been documented more directly in numerous tight epithelia by electrical methods[17-19]. (It has yet to be demonstrated for a leaky epithelium.) Stimulating the pump with CO_2 increases apical Na$^+$ permeability, while inhibiting the pump by metabolic poisons or by the specific pump inhibitor ouabain reduces apical Na$^+$ permeability. The effect makes sense teleologically: shutdown of basolateral Na$^+$ exit leads to shutdown of apical Na$^+$ entry, protecting the cell against osmotic lysis. But this result, however desirable, is paradoxical. The simplest expectation from shutdown of basolateral Na$^+$ exit would be a rise in cell Na$^+$, tending to increase apical Na$^+$ conductance because of the higher concentration of the conducting ions. In fact, apical Na$^+$ conductance decreases.



Evolution of the Koefoed-Johnsen and Ussing model of epithelial transport. *a*, As originally postulated in 1958. *b* and *c*, The present form of the model applied to tight (*b*) and leaky (*c*) epithelia.

The converse type of cross-talk, a regulation of the basolateral membrane by the apical membrane, has just been demonstrated by two recent papers. In the tight epithelia toad and frog urinary bladders, Davis and Finn[3] showed that blocking the apical Na$^+$ channel with amiloride decreases basolateral K$^+$ conductance as rapidly as apical Na$^+$ conductance. In the leaky epithelium Necturus small intestine, Gunter-Smith, Grasset and Schultz[4] found that addition of the sodium ion's transport partners, alanine or glucose, to the luminal bathing solution rapidly increases apical conductance, then more slowly increases basolateral conductance. Furthermore, there is now extensive evidence of feedback within the basolateral membrane: an increase in activity of the basolateral Na$^+$–K$^+$ pump goes hand-in-hand with an increase in basolateral K$^+$ conductance[4,15,19,20]. What remains unclear is whether this basolateral K$^+$ conductance is part of the pump itself, or whether it arises from a separate channel. It is also uncertain whether a rise in cell Na$^+$ is the principal means by which increased apical conductance leads to increased basolateral pumping, since searches for the expected rise in cell Na$^+$ have yielded conflicting results[21-23].

What signalling agent is involved in these feedback circuits? The leading contender is changes in intracellular Ca^{2+}, normally maintained at a low concentration by countertransport of Na$^+$ versus Ca^{2+} at the basolateral membrane[24-26]. In other words, Na$^+$ entry downhill into the cell at the basolateral membrane drives the uphill extrusion of Ca^{2+}, so that reduction of the Na$^+$ downhill gradient through a rise in cell Na$^+$ would lead to a rise in cell Ca^{2+}. To explain the observed feedbacks, one would also have to assume that raised cell Ca^{2+} increases basolateral K$^+$ conductance but decreases apical Na$^+$ conductance. It remains to be determined whether physiological changes in cell Ca^{2+} caused by changes in cell Na$^+$ do produce these conductance changes.

All these examples of transcellular cross-talk have involved Na$^+$ transport. A possible example for another ion has emerged from studies by Machen and colleagues[27-29] in gastric mucosa, where increased Cl$^-$ transport is associated with increased transepithelial conductance. The effect appears to involve increases in basolateral K$^+$ conductance and in apical Cl$^-$ conductance.

Similar interactions between conductances may also appear in isolated cells, as exemplified by an effect of Na$^+$ entry on K$^+$ conductance in hepatocytes[30]. However, it is proving harder to discover feedback links in isolated cells, where the interacting conductances coexist in the same membrane, than in epithelia, where they are conveniently segregated in opposite membranes.

Comparison of Fig. 1*a* with Fig. 1*b* and 1*c* makes clear that the KJU model has evolved considerably since it was proposed in 1958. In tight epithelia (Fig. 1*b*), the pump is now believed to be electrogenic instead of neutral, and three feedback mechanisms have been added. In leaky epithelia (Fig. 1*c*) a major parallel pathway and one feedback mechanism have been recognized, and the apical entry step is now considered not to be of Na$^+$ alone. These developments illustrate why, in the past two decades, philosophers of science have talked less about the verification or falsification of models, and more about the evolution of models within a continuous framework (for example, the paradigm or disciplinary matrix of Kuhn, the research programme of Lakatos)[31].

But the history of the KJU model also illustrates that, even at a given stage, a model's acceptance does not depend solely on its verifiability, or on its success in surviving attempts at falsification. Through much of the 1960s and the early 1970s many experimental findings argued against even the basic premises of the KJU model[32-38]. Why did the model nevertheless remain widely accepted through this period? The fact that today's evidence happens to support the model does not resolve the dilemma, for this outcome was not foreseen from yesterday's evidence.

Several factors may have contributed to this paradox. Although the KJU model conflicted with some important findings, there was never a serious alternative that could begin to resolve these conflicts while matching the successes. Although there

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were doubts about the correctness of the KJU model, its use continued to suggest numerous experiments that yielded significant results. There was concern that the error was not in the model but in the contrary findings: for instance, in estimates of intracellular ion concentrations by indirect chemical means, superceded recently by direct measurements through ion-specific microelectrodes.

Finally, the model was beautiful, simple and reductionist: it made epithelial phenomena emerge from established properties of isolated cells. In contrast, the findings conflicting with the model were complex, intellectually unappealing and unconnected to work on isolated cells. Given a choice between what seemed to be a beautiful model and ugly findings, physiologists preferred the model. Now, many of the ugly findings that conflicted with the model can be explained by our newly-gained understanding of cross-talk. Beauty and truth are once more united! □

Trypanosoma cruzi: signals for transformation

from F.E.G. Cox

Trypanosoma cruzi is the causative organism of Chagas' disease, a disease that affects between 12 and 24 million people in South and Central America and causes considerable mortality and morbidity. The trypanosome is transmitted by numerous species of blood-sucking reduviid bugs. Trypanosomes in the trypomastigote form are taken up by the bug when it feeds and transform into epimastigote forms in the anterior part of the insect's gut. They then multiply by binary fission, building up vast numbers. Transmission becomes possible when the epimastigotes transform back into trypomastigotes again and pass into the hindgut where they are voided with the faeces to enter the victim through abrasions in the skin. The bugs live for long periods and, having become infected, remain infective for life, so providing a continuous threat to all those who might subsequently be bitten.

The signals that trigger these transformations have not yet been definitely identified but it seems certain that the membrane surface of the epimastigote form recognizes signals provided by the intrinsic or extrinsic contents of the insect's gut. The membrane surface is very complex and contains polysaccharides, lipopolysaccharides and glycoproteins. The carbohydrate components can be identified using lectins[1], and peanut butter agglutinin can be used to extract galactose-terminal glycoproteins[2]. Monoclonal antibodies can also be used to identify glycoproteins[3]. The tools with which to study the signals responsible for transformation in the bug are therefore available.

It has been suggested that the lectins already identified in the insect gut might regulate the morphogenesis of the trypanosome[4]. One of the molecules possibly involved has now been identified[5] in a letter that has just appeared in *Nature* (Sher, A. & Snary, D. **300**, 639; 1982). Epimastigotes do not readily transform into trypomastigotes (a problem that has also troubled workers using African trypanosomes) and Sher and Snary[5] devised a technique whereby epimastigotes enclosed within Millipore chambers were transplanted subcutaneously into mice, left in position for 7 h, withdrawn and transferred to an appropriate culture medium. In these conditions, epimastigotes transform into trypomastigotes, a change that does not occur if the *in vivo* 'programming' is not carried out. Using this technique, Sher and Snary were able to investigate the inhibitory effects of six monoclonal antibodies against epimastigote surface components and one lectin, wheat germ agglutinin. The substances to be tested were introduced into the culture media containing 'programmed' epimastigotes and the proportion transformed calculated.

The experiments showed that only one of the monoclonal antibodies inhibited transformation from the epimastigote to the trypomastigote form. The antibody had already been shown to be directed against a cell-surface glycoprotein with a molecular weight of 72,000 (ref.3). It does not affect the longevity or motility of the epimastigote, only its ability to transform into a trypomastigote. The lectin had no inhibitory effect. The results suggest that in the midgut of reduviid bugs there exists a substance, or substances, which reacts with the 72,000-molecular weight glycoprotein on the epimastigote surface and inhibits transformation into the trypomastigote form. Whether or not this is one of the lectins previously described has yet to be determined.

The significance of these findings is that they provide a clue as to why epimastigotes are inhibited from transforming into trypomastigotes. The inhibition probably serves two purposes — to accumulate epimastigotes in the midgut until such time as a relatively large proportion can transform into trypomastigotes, so ensuring the infection of a new host; and to retain a number of untransformed epimastigotes for later transformation and infection. Parasites need to be retained in the midgut because the nutritional conditions are more favourable there than in the hindgut.

From an epidemiological point of view the findings are very interesting. Many bugs can transmit *T. cruzi* but not all do so equally well and it is possible that the potential to act as a reservoir of infection also varies. Susceptibility and the ability to transmit the infection may be tied up with the interactions between surface molecules and insect products and as there are quantitative and qualitative differences between the surface components of different strains of *T. cruzi*[6] it may well be that a full understanding of Chagas' disease will depend on developments at the molecular level. The findings may also be significant in other situations; in leishmaniasis, for example, lectins have also been shown to bind to the surfaces of the parasites and the capacity to bind to vary between species and strains[7]. Monoclonal antibodies, at present largely the tool of immunologists, may become equally important to epidemiologists. □

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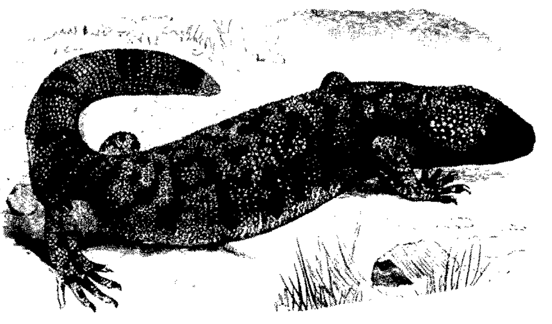

THE SONORAN HELODERM

(*Heloderma suspectum*)

At least one form of lizard is endowed with the faculty of producing a poisonous bite. The possessors of these formidable weapons of defence are the members of the genus *Heloderma* of naturalists, one species of which was long ago described from Mexico under the appropriate name *Heloderma horridum*. This lizard has not only grooved teeth, after the manner of many of the poisonous serpants, but likewise highly developed salivary glands, which issue at the bases of the teeth with the evident purpose of carrying poisonous saliva into the grooves.

From *Nature* **27**, 153; December 14, 1882.

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Why is the stratosphere dry?

from Reginald E. Newell

MORE than 30 years ago Brewer and Dobson[1,2] suggested that the stratosphere is dry because air enters from the troposphere only in the vicinity of the cold tropical tropopause. They proposed that the tropical tropopause acts as a cold trap for moisture, limiting the stratospheric moisture to saturation mixing ratio values at the trap temperature. Working backwards from middle-latitude observations the required cold trap temperature is about −84°C. The general theory Brewer and Dobson still holds but the details of the actual troposphere-to-stratosphere transfer are not understood. Does the transfer occur by a steady rising motion or is it part of the active cumulonimbus convection occurring in the tropics? A series of papers[3-9] published in *Geophysical Research Letters* this year gives some insight into the processes involved.

Brewer and Dobson based their theory on frost-point measurements made from aircraft over England, which showed that whatever the conditions in the troposphere, the stratospheric water vapour mixing ratio dropped sharply just above the tropopause to values close to 2 μg of vapour per gramme of air, only one ten-thousandth of the value just above the tropical sea surface. Mastenbrook[10] subsequently adopted Brewer's frost-point hygrometer for balloon use and found similar low mixing ratios in the tropical stratosphere with a seasonal variation generally in phase with the cold trap temperature.

The recent papers in *Geophysical Research Letters* provide the first results from an exploration of the tropical tropopause region by the NASA high-flying U-2 aircraft which was based in Panama during September 1980. The aircraft was equipped with an array of modern instrumentation and made a series of flights to measure temperature, water vapour, aerosols, ice crystals and other atmospheric trace constituents. The results show a water vapour minimum at about 19 km, a few kilometres above the temperature minimum at the tropical tropopause, with mixing ratios down to about 2.2 μg g^{-1}, significantly lower than the saturation mixing ratios ($\sim$ 3.8 μg g^{-1}) at the observed tropopause temperatures (around −80°C). The air at 19 km had clearly not entered the stratosphere through the tropopause at Panama. The water vapour was measured with two instruments, a new Lyman-α photodissociation hygrometer[8] and a more conventional broad-band IR radiometer[9], and both showed the same general findings.

Aerosols with radii greater than 0.03 μm were collected by a wire impactor and examined in the laboratory with a scanning electron microscope[6]. It was found that the concentration of small particles diminished and that of larger particles increased with height above the tropopause. The small particles seem to be introduced into the stratosphere by convective activity, though where and when the injection occurs is not known; coagulation seems to be the main mechanism of loss.

The U-2 pilot was bold enough to penetrate the cirrus anvil on several occasions (a somewhat risky procedure as it is hard to see from above where there might be convective updrafts penetrating the lower part of the anvil) and a probe was used to record two-dimensional shadow images of ice crystals in the size range 40–2,500 μm (ref. 7). In addition, an active cavity laser spectrometer was used to measure the size distribution of aerosols in the 0.1–0.3 μm size range. Inside the anvil there was a particular abundance of aerosols having a radius of 0.17 μm which Knollenburg and co-workers think is due to new nucleation and growth of solution droplets[7]. This is just one item from the large amount of new data on anvil particles that was collected in Panama.

To accompany the aircraft measurements, enhanced IR photographs of the tropical clouds were produced from the Atlantic Geosynchronous Satellite[4]. These showed the time evolution of the convection and the large extent of the anvils, although there were only a few points where the equivalent temperature was lower than −82°C, in agreement with the aircraft observations.

It had been thought that the moisture in the rising air would condense out to form cirrus cloud and the ice crystals would be left in the troposphere[1,2]. Danielsen[5] has examined the new data with this view in mind and interprets it in terms of what he calles 'a dehydration engine'. He describes how a rising cumulonimbus cloud can overshoot the level of zero buoyancy and the anvil form from air that has descended after some mixing with warmer and drier air of the lower stratosphere. The anvil base extends far beyond the parent convective cloud, as shown by the satellite IR images, and is heated by the interception of upwelling IR radiation. The satellite images and aircraft data together suggest an IR emissivity close to unity for the anvil top and this region cools by radiation to space. Static instability is thus maintained in the anvil by radiation and there is a turbulent heat flux from the base to the top for balance. Because the anvil base is about 10°C warmer than the top and saturation vapour pressure increases with temperature, there is a vertical gradient of moisture and an upward moisture flux is produced by the turbulent transport. Near the top of the anvil there is supersaturation, perhaps even with respect to water according to the observations, and ice crystals grow and fall relative to the air, providing the water sink for the system. The engine mechanism yields a vertical temperature profile with two minima and an intermediate warmer region due to anvil subsidence; the two minima are observed in the flight data. Furthermore, calculations of IR cooling from the ice crystal data and from the IR radiometer data[7] show cooling rates near the top of the anvil of up to 15°C per day, giving further support to Danielsen's engine concept.

The finding that the water vapour mixing ratio is at a minimum near 19 km, well above the tropopause, and has values smaller than saturation values at the tropical tropopause suggests that air enters the stratosphere in a region where the tropopause is colder than that at Panama. Considerations of the world-wide distribution of temperature indicate that the tropical western Pacific is a region where the exchange might occur, particularly in the December–February season[5,8,11].

That possibility, and Danielsen's concept of a 'dehydration engine', could be tested by carrying out a similar experiment in that region, and with more extensive radiative measurements it should be possible to check Danielsen's predictions of a radiatively produced instability. Even in the western Pacific convection can only dehydrate the air just above the tropical tropopause. The dry air seen at 19 km at Panama must have risen by a gentle rising motion that would accompany radiative heating of the region above the convection and this process would take place while the air moves in a longitudinal direction.

That there is a minimum in the mixing ratio at all is perhaps surprising as the early ideas were that the mixing ratio should be constant with altitude above the tropopause. Indeed, an observation of increased mixing ratio at higher altitude was often explained as contamination[10]. But it is now

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thought that significant quantities of water vapour may be formed at higher altitudes by chemical and photochemical reactions which destroy methane[12], another gas that passes into the stratosphere through the tropical tropopause. In fact the results of these experiments will have a general applicability to the transfer of all tropospheric trace gases into the stratosphere[13]. For most of these the transfer is not limited by a physical property, such as the variation of saturation vapour pressure with temperature that limits the stratospheric water content, and if fresh tropospheric air is sampled above the tropopause in a region such as the western Pacific it should be possible to detect relatively short-lived tropospheric species.

Bright future for liquid electrolyte solar cells?

from A. Hamnett and S. Dennison

THE past decade has seen major efforts to develop photoelectrochemical devices that directly convert light to electricity. The devices are based on the junction between a semiconductor and a liquid electrolyte, but problems of corrosion and efficiency have bedevilled their successful development into a technology that could rival the more familiar solid-state p–n junction solar cells, where efficiencies of more than 20 per cent have been attained. The superiority of the solid-state p–n device may, however, appear a little less assured with a report in this issue of *Nature* by C. Gronet and N. Lewis of Stanford University (see p.733) who describe a photoelectrochemical cell (PEC) based on n-GaAs$_{1-x}$P$_x$ in acetonitrile and using as redox couple the ferrocene-ferrocinium system. The cell has an operating efficiency of 13.2 per cent in natural sunlight, a figure considerably in excess of any previously reported from a non-aqueous cell.

The essential principles of the liquid-junction photovoltaic device can be understood with reference to the figure. If an n-type semiconductor is placed in a concentrated electrolyte and subjected to a positive bias, the region of the semiconductor near the surface becomes depleted of electrons. When light of sufficient energy is absorbed in this 'depletion layer', the electric field is able to separate the resultant photogenerated electron (in the upper or conduction band) from the positively charged vacancy or 'hole' left behind in the lower or valence band. The direction of the field is such as to drive the 'hole' to the surface, where it is transferred to the reduced form, R, of a suitable redox couple O/R. The electron, meanwhile, is transported around the external electrical circuit, performing work e_0V, finally reducing O at the metal counter-electrode. No net chemical change occurs in the cell, but light energy $h\nu$ is converted to electrical work e_0V. The figure makes it clear that there will be two limiting cases; the open-circuit voltage V_{oc}, which is the maximum voltage obtained at zero current, will be approximately the difference between the potential corresponding to the conduction band edge and the redox potential of O/R. By contrast, the maximum current is obtained when the two electrodes are short-circuited. Between these two extremes, there will be a current-potential region where useful work can be obtained; owing to the spectral distribution of the solar spectrum, there will also be an optimal bandgap for the semiconductor which is $\sim$ 1.4 eV, though bandgaps between 1.0 and 1.8 are likely to be effective.

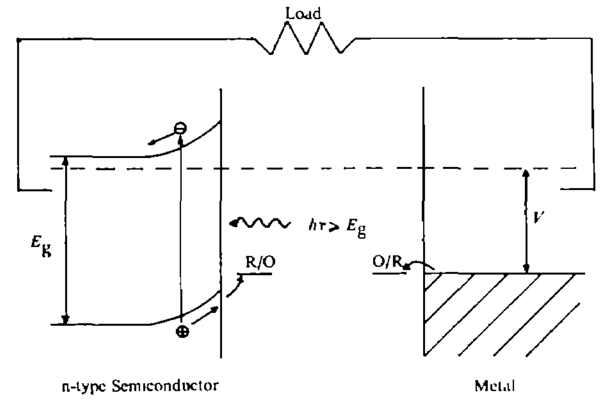
Although the principles of this cell have been known for some years, it has proved extremely difficult to build practical devices based upon them. Four problems have arisen that have proved especially obdurate; of these, photocorrosion is the most serious and occurs when some of the photogenerated holes arriving at the surface become diverted from the desired reaction (conversion of R to O) and instead oxidize the surface of the semiconductor. To avoid this, a very fast rate constant for the oxidation of R is necessary, which minimizes the concentration of holes at the surface. A second related problem is that holes at the surface may also be diverted to electronic energy levels that are localized in the surface region — so-called 'surface states'. If these lie near the conduction band edge, they may in turn capture an electron from the conduction band, the net effect being the degradation of the light energy to heat. In aqueous solutions especially, surface states near the conduction band edge are very common and are thought to be due to protons. Much effort and ingenuity has gone into attempts to mitigate the deleterious effects of these states.

Closely related to this is the problem of grain-boundary recombination; ideally, the semiconductors used in such photovoltaic devices should be polycrystalline rather than single-crystal materials. From the economic standpoint, polycrystalline materials are enormously advantageous, but solid-state p–n junctions using them have proved extremely difficult to fabricate. This is because such junctions are made by taking a slice of n- or p-type material and diffusing into it, from the surface, impurity atoms that dope it locally in the opposite sense. Since diffusion down grain boundaries is far more rapid than diffusion in the bulk, it is prodigiously difficult to control the width of the junction in polycrystalline samples. No such fabrication problems arise in the liquid-junction photovoltaics described above, but recombination of carriers at the grain boundaries can cause a severe drop in operational efficiency.

The third problem is the kinetics of the O/R couple. We have seen that oxidation by holes must be rapid to compete with photocorrosion and surface-state capture processes, but additionally the kinetics of reduction of O to R must also be fast to reduce the overpotential at the metal cathode. In effect this means that the O/R couple must be highly electrochemically reversible.

Finally, strong chemisorption of the redox couple onto the electrode can occur. If both R and O strongly adsorb on the semiconductor surface, conversion of R to O will alter the charge balance at the interface. This in turn will reduce the electric field in the depletion layer, reducing the rate at which photogenerated holes can be

The design of the liquid-junction photovoltaic device. O/R is the redox couple.



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transported to the surface. A steady state is set up in which the change in the depletion layer charge is determined by the relative rates at which holes can be fed from O_{ads} to R in solution and the rate at which they can be supplied by the electrode. If the former rate is slow, the overall quantum efficiency will be very low.

In attempting to overcome these problems, a great deal of effort has been invested in pursuing a detailed understanding of the fundamental surface processes on semiconductors in contact with aqueous and non-aqueous electrolytes. In parallel with this work, novel methods of chemically modifying the surface to improve the quantum efficiency have been explored, and a large amount of phenomenological information gathered. Heller's group at Bell Laboratories has been especially active in this field, and has made considerable progress in reducing the effects of corrosion and surface-state recombination in cells based on aqueous electrolytes. The technological price has, however, been severe; water is a highly corrosive solvent, partly because of its excellent solvating properties, and complex measures for protecting the surface, involving various chemisorbed metal ions, have had to be developed.

These technological difficulties have had a rather discouraging effect on aqueous photovoltaic work. In an effort to avoid some of the problems, several groups around the world have begun programmes of research into non-aqueous electrolytes. Initially, behaviour was found which suggested that chemisorption of the redox couples used was very common, and it was suggested that these cells would therefore have an intrinsic limitation to their efficiencies. However, it now appears from the work of Gronet and Lewis, reported in this issue, that no such intrinsic limitations exist. By using n-type $GaAs_{1-x}P_x$ electrodes and extremely straightforward cell design, they have obtained efficiencies of over 13 per cent. This is a remarkable result especially as, by the authors' admission, the cell is quite unoptimized. There can be little doubt that this result will lead to a considerable reassessment of liquid-junction photovoltaic devices and the obviously urgent task is now to investigate the use of polycrystalline electrodes to see whether the real advantages of such cells can be realized. □

Keratinocyte culture club

from Fiona M. Watt

In recent years new techniques for the cultivation of epidermal cells (keratinocytes) have generated enormous interest among clinicians and biologists alike. Dermatologists are attracted by the possibility that keratinocytes from patients with skin disorders may behave abnormally *in vitro*. Given the clinical importance of squamous cell carcinomas, tumour biologists are discarding fibroblasts in favour of keratinocytes for many *in vitro* models of transformation and carcinogenesis. Keratinocytes appeal to the cell biologist too: as a system for studying terminal differentiation and population kinetics; for investigating basement membrane synthesis and the structure and function of keratin filaments. Scientists with these diverse interests gathered recently at a symposium on keratinocyte biology* which turned out to be lively and thought-provoking.

Methods of cultivating keratinocytes seem to proliferate more rapidly than the cells themselves, but generally fall into two categories: first, relatively simple systems for serial passage of cells, in which some of the properties of intact epidermis are retained; second, more elaborate attempts to reconstruct true epidermis in primary culture by approximating a normal epidermal environment. Of the first group, the most successful is undoubtedly that of Rheinwald and Green (*Cell* **6**, 331; 1975), in which human keratinocytes can be grown for many generations on a feeder layer of 3T3 cells. There have been several attempts to eliminate the requirement for feeder cells and a new chemically defined medium for human keratinocytes seems quite promising (S. Boyce, University of Colorado). It is harder to persuade mouse keratinocytes to proliferate *in vitro*, and consequently most cultures are seeded at high density. A low level of calcium ions (< 0.1 mM) enhances proliferation of newborn mouse epidermal cells (H. Hennings, NCI), but as this prevents stratification, normal terminal differentiation, in which a steady state is achieved between basal cell division, suprabasal differentiation and cell shedding, is not observed.

The histological appearance of cultured epidermis can be improved by growth on a 'dermal equivalent' — a collagen gel containing fibroblasts (E. Bell, MIT). Further attempts to mimic the normal environment of epidermal cells include culturing at an air-liquid interface, feeding cells from below and providing a growth substrate of basement membrane components, all of which can improve the differentiated morphology of primary cultures (M. Prunieras, Fondation Rothschild; I. Mackenzie, University of Iowa).

In order to analyse normal differentiation or to define abnormalities in disease, it is essential to have good biochemical markers of keratinocyte differentiation; morphological criteria are not enough. Late in terminal differentiation an intracellular envelope of protein cross-linked by transglutaminase is formed. So far, two envelope precursors have been identified and solemnly named — involucrin (R. Rice, Harvard University) and keratolinin (not to be confused with keratohyalin) (L. Peterson, Oregon Health Sciences University). While it is still too early to say how many envelope precursors there are and how they are related, individually they provide good markers of terminal differentiation. Lectin binding by keratinocytes changes during differentiation, reflecting altered membrane glycosylation (P. Elias, VA Medical Center, San Francisco; F. Watt, Kennedy Institute). Such changes in keratinocyte surface properties might influence cell recognition and adhesion and could account for the sorting out of differentiating cells during stratification. Monoclonal antibodies to keratins reveal patterns of keratin synthesis characteristic of different stages of differentiation (T.-T. Sun, New York University). With elegant new STEM and linear mass density studies on keratin filament structure (P. Steinert, NCI) and with amino acid sequences of individual keratins obtained by gene cloning (E. Fuchs, University of Chicago; D. Roop, NCI), the biological significance of keratin heterogeneity may at last become clear.

Certain skin disorders, such as congenital ichthyosis, are characterized by distinct ultrastructural abnormalities which may continue to be expressed *in vitro* (I. Anton-Lamprecht, Institute for Ultrastructure Research, Heidelberg). However, good morphological criteria of disease are unusual in keratinocyte cultures, and many studies of potential clinical relevance are hampered by failure to exploit the known biochemical markers of normal terminal differentiation instead. This will surely change before long: it is exciting to learn, for example, that human cervical displasia is correlated with a decrease in involucrin synthesis, as this should be amenable to analysis in culture (R. Rice). □

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*'Biology of the Keratinocyte In Vitro', 32nd Annual Symposium on the Biology of Skin, was held at Gleneden Beach, Oregon on 10-14 October 1982.

Corrigendum

In the *News and Views* article 'Transformation and gene organization in *Drosophila*' (*Nature* **300**, 15; 1982), the work on cell division mutants (penultimate paragraph) was attributed solely to B. Baker's La Jolla laboratory. Since its inception, this research has in fact been done in collaboration with the laboratory of M. Gatti (Rome).

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REVIEW ARTICLE

Changes in the solar constant and climatic effects

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Spacecraft measurements have established that the total radiative output of the Sun varies at the 0.1–0.3% level. The observed fluctuations are well modelled as radiative deficits proportional to the area of the solar disk covered by sunspots. Historical records of projected sunspot areas allow an accurate reconstruction of these short-term fluctuations over the past century. Such changes can be expected to perturb the terrestrial surface temperature by a fraction of a degree centigrade and probable evidence of this solar-induced signal has been found. The effect, though important in terms of understanding the climate system, is too small to be significant in practical weather or climate predictions.

RADIATION from the Sun is the dominant source of energy input to the Earth's atmosphere. Solar energy, coupled with the rotational inertia of the spinning planet, drive the circulation patterns of oceans and air that are the basis for all the phenomena known as weather and climate. Regional weather patterns may result from local perturbations of geographical, orographic, or other origins, including anthropogenic ones, but all of these processes depend on a fundamental flux of solar energy. The equilibrium temperature of the Earth's surface and seas is ultimately determined by a balance between solar radiation absorbed by the Earth, primarily at visible and near IR wavelengths, and long-wave radiation that is re-emitted to space. Should the total solar input vary as a persistent trend, the surface temperature of the Earth will in time respond in a direct and predictable fashion: global cooling in response to decreased solar radiation, and warming following an increase. The diurnal variation of cold nights and warmer days, and seasonal patterns from winter to summer attest to the role of insolation as the principal temperature control.

Such reasoning has prompted innumerable attempts at identifying the signature of solar variability in climate records. What is surprising is how difficult it has been to find any unequivocal evidence of any significant forcing by known solar variability[1,2]. Meadows[3] has traced the spotted and often confused history of such attempts, most of which were statistical tests of supposed connections between observed solar activity and various meteorological parameters. He adopted an earlier estimate[4] that "Solar activity does not produce a significant variation [upper limit of 0.1%] in the value of the solar constant". With this exclusion, any significant Sun–weather effects depended on indirect or 'trigger' mechanisms, which might result, for example, from perturbations in the energetically weak solar wind on the Earth's upper atmosphere and possible subsequent tropospheric response. Meadows concluded that: "If [an acceptable mechanism] could only be defined, the whole controversial field might be rapidly translated into the realms of high respectability".

We discuss here recent evidence for measured variations in the solar luminosity which provide a direct and simple mechanism for associated climatic change. Such variations, though small in amplitude, constitute a significant breakthrough in solar–terrestrial physics: for the first time a form of solar variability has been identified that is sufficiently energetic to alter directly the basic radiation balance of the lower atmosphere. Moreover, a wholly consistent response in surface temperature has already been (independently) identified[5], lending credence to the reality of the connection. The resultant changes in surface temperature are very small—no more than a few tenths of 1 °C. But the existence of a probable response opens the door to the possibility of larger effects, due to longer and more important changes in the bulk radiative output of the Sun.

Observational evidence for solar constant changes

Precision measurements of the total solar flux, or 'solar constant', *S*, have long been desired; they have become possible on a continuous basis only in the present decade with sophisticated radiometers in Earth orbit. Langley had foreseen the need to rise above the atmosphere 80 yr ago when he called for the establishment of an elevated solar observatory[6]: "The Earth's temperature and the life of its inhabitants, both animal and vegetable, depend on the solar radiation. Yet we confess that even at this late day we do not know, with any certainty, what the total amount of solar radiation is, whether it is constant or variable, or what effect upon terrestrial life and temperature a given change in it would produce. Our ignorance of these fundamental things is largely, though not wholly, due to the variability of our own atmosphere, which prevents us from studying that of the Sun".

In 1881, Langley had attempted a precision measurement of the solar constant from the top of Mt Whitney, 14,495 ft. elevation. Subsequently, he and Abbot for many years tried to measure the solar constant from mountain tops but their measurements were still limited by uncertainties in atmospheric transmission to about ±1% [7]. In 1980, near the peak of the

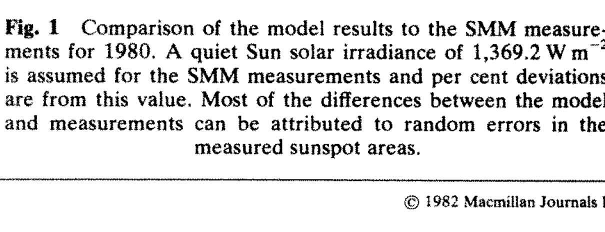

Fig. 1 Comparison of the model results to the SMM measurements for 1980. A quiet Sun solar irradiance of 1,369.2 W m^{-2} is assumed for the SMM measurements and per cent deviations are from this value. Most of the differences between the model and measurements can be attributed to random errors in the measured sunspot areas.

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solar activity cycle, the SMM spacecraft succeeded in accumulating nine months of continuous measurements of the solar constant with an unprecedented internal precision of 0.01% [8]. In these data was clear evidence of changes at the level of 0.1–0.3%, about an order of magnitude lower than the threshold of previous, ground-based measurements. A similar radiometer on the Nimbus 7 spacecraft[9,10] confirmed that these excursions, lasting 1 to 2 weeks, were depletions in the total output of the Sun. With these data the possibility of a rigorously constant *S* was unambiguously disproven for the first time.

Theoretical understanding of solar constant changes

The continuous solar constant measurements accumulated by the SMM in 1980 are shown in Fig. 1, as daily averages. Error bars are approximately the width of the line used to trace the record. It is obvious that the variations of *S* seen by the SMM are not those of a white noise spectrum, but are well approximated by a steady background with minor rises and major, superposed dips of varying amplitude. The time series of Fig. 1 suggests a process by which *S* is depleted over time scales of days to weeks without a compensating emission process. Moreover, initial analysis of the SMM data established the fact that the major dips were always coincident with the passage of large sunspot groups across the solar disk.

Sunspots are localized regions on the solar surface which appear dark in relation to the background solar photosphere (Fig. 2). The umbra, or dark central region of a sunspot, has a temperature of about 4,000 K, some 1,800 K or 30% cooler than the adjacent photosphere. A sunspot penumbra has a typical temperature of about 5,400 K.

Sunspots are regions of intense, concentrated magnetic field. These strong magnetic fields inhibit the local, vertical, convective transport of energy, leading to a lower, local radiative flux and a corresponding reduction in local temperature. It has long been suspected that the presence of sunspots, which are known to follow an 11-yr recurrence cycle, could modulate the Sun's total radiative output. In serious question, however, has been whether solar activity would decrease[11] or increase[12,13] the total solar flux, because dark sunspots are always accompanied by bright (hotter) facular regions in the photosphere and chromosphere. A third and equally tenable hypothesis was that a detailed balance in *S* would ensue between sunspot depletion and facular emission, resulting in a rigorously invariant solar constant. The SMM and Nimbus measurements establish that only a small fraction of the energy blocked by sunspots is balanced by immediate enhanced emission from bright features such as faculae and plages[8,14–16]. On time scales of months or years, there is no detailed balance. As we shall see, this fact has practical consequences for the effect of solar variability on the Earth's temperature.

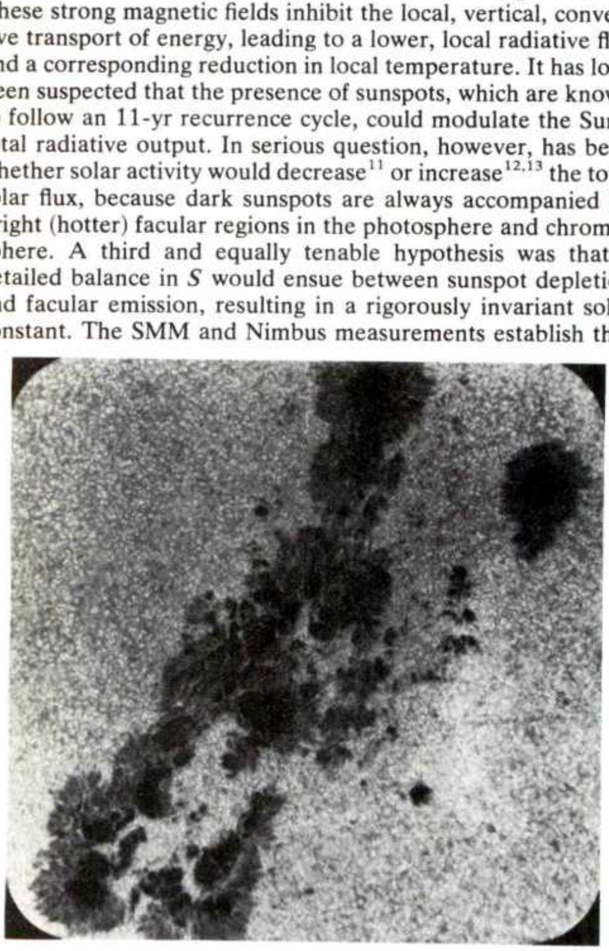

Fig. 2 A large sunspot group showing dark umbral and intermediate penumbral regions contrasted against the brighter surrounding photosphere. (Sacramento Peak Observatory, Association of Universities for Research in Astronomy, Inc.)

The basis for these important conclusions is the success of simple, blocking models in replicating the observed pattern of fluctuations in *S* that were observed with the SMM and Nimbus spacecraft (Fig. 1) using daily, observed values of projected areas and positions of sunspots made by ground stations. The symmetric, gaussian shapes of the major dips are readily explained as the result of spherical projection: a sunspot of fixed size will block more of the apparent area of the solar disk as solar rotation carries it towards the Sun's central meridian. Furthermore, the magnitude of the observed depletions in *S* are well duplicated by measured sunspot areas and canonical values of umbral and penumbral contrasts.

Schatten *et al.*[17,18] have endeavoured to attribute considerably more of the observed fluctuations in *S* as due to facular brightening, thereby implying a detailed balance in the total solar flux. This divergent interpretation is challenged by other, independent studies of the same spacecraft data[8,14,15,16,19–21]. As explained elsewhere[22,23], the discrepancy may be due to the failure of studies implying detailed balance to make use of actual, daily observations of sunspot and facular areas or to utilize the full SMM solar constant record. When this is done a better fit and a more accurate interpretation is obtained. The difference in terms of the storage of radiative flux within the Sun and in terms of potential climate effects is fundamental.

The model for sunspot blocking used in Fig. 1 is based on daily measured positions and areas of sunspots and on standard umbral, penumbral and facular contrasts of −0.75, −0.25 and +0.03 respectively[14]. Also included is the well-known effect of limb-darkening. The brightness of the apparent disk of the Sun decreases towards the edge, or limb; as a consequence sunspots near the limb have lower contrast relative to the background Sun than they do near the centre of the disk. The amount of irradiance blocked by a sunspot therefore decreases as it moves away from the apparent centre line, seen from the Earth, due to a combination of geometrical foreshortening and limb darkening. The model used in Fig. 1 also assumes that the blocked radiation is never released. In time it must be, but with the data now available it is possible only to conclude that the blocked energy does not reappear in recognized form for time scales of at least months, and probably a year. Continued measurements should help to refine the estimate of effective storage time. The notion of a frustrated Sun, with stored, and gradually released energy from remembered sunspots is not an altogether unreasonable one. Blocked, radiative energy can be stored in mechanical form in the lower convection zone with a relaxation time of up to 10^5 yr, (refs 24–26) during which time it will only slowly seep out. For purposes of comparisons with SMM or Nimbus data a storage time of this length is almost infinity.

The remarkable fit of SMM observations with simple, sunspot blocking models allows us to take two further steps. We can now predict the short-term excursions in *S* (should climatologists want them) a few days in advance, based on easily obtained measurements of sunspot areas and positions and the known rotational properties of the Sun. By the same technique we can also reconstruct the past history of daily fluctuations in *S* from archived sunspot data. Precise records of sunspot areas and positions exist for more than 100 yr. Figure 3 shows reconstructed, daily records of *S* for four sample years as they were generated from historical sunspot records[14]. The 108-yr reconstruction shows the expected signature of the 11-yr sunspot cycle, with greater accumulated depletion during years of enhanced sunspot numbers.

These predictions and reconstructions tell all we know with certainty of solar constant excursions. But there may well be more to the story in the form of longer, possibly independent

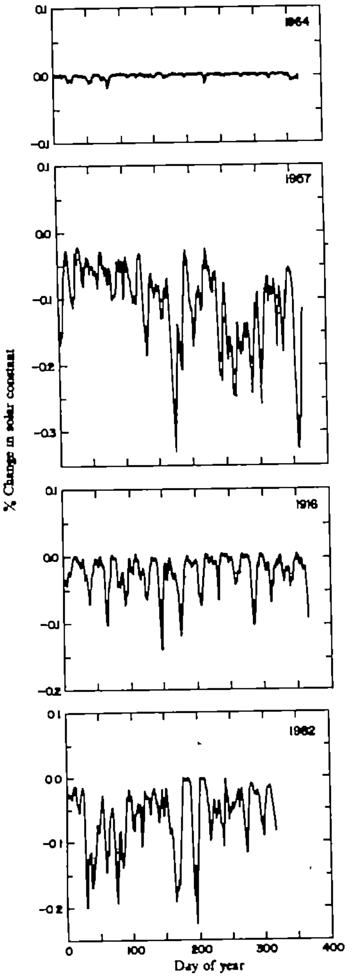

Fig. 3 Daily solar constant values reconstructed from sunspot and facular area records for sample years of low activity, high activity and recurrent, moderate activity with a similar reconstruction for 1982, to 14 November.

trends. Such trends, if they exist, could be of greater climatic impact, through persistence, than the short-term sunspot blocking effects noted above. The existence of one such possible trend, an apparent decrease in S of ~0.02–0.04% yr^{-1}, has already been noted in the Nimbus[27] and SMM[28] records. The model shown in Fig. 1 would predict a downward trend of about this amount in the 1979–82 period due to an averaged increase in sunspot areas during this time, even though sunspot numbers were simultaneously decreasing. It is probably too early, however, to determine whether the measured trend is real, and, if real, how much of it is due to accumulated sunspot blocking or some other effect.

We may sum up the recent discoveries concerning the solar constant as follows:

(1) The solar constant varies.

(2) The fluctuations are almost wholly explained by the simple mechanism of sunspot blocking, by which S is reduced in direct proportion to the fraction of the apparent solar disk that is covered by sunspots.

(3) Excursions in S during high solar activity are at most 0.5%, and more generally 0.1% or less, maximizing in each case when large sunspot groups are near the central meridian.

(4) Excursions of this nature persist throughout the period of visibility of the spot—for 14 days or less.

(5) The radiation blocked by sunspots does not immediately reappear where it can be seen or sensed; it is in some way stored within the Sun, to seep out much more slowly as an integrated increase in radiative flux.

(6) Photospheric and chromospheric faculae (surface regions brighter than the background disk) play at most a minor role in modulating the instantaneous value of S.

Evidence for a solar cycle effect in temperatures

A possible, 11-yr signal due to sunspot effects has long been sought in both regional and globally-averaged surface temperature records[29–34]—a search that has origins in ancient weather lore[35] and in literature[36,37]. The lack of any clear success in these attempts almost certainly implies that any globally distributed, coherent variation due to sunspot-cycle forcing must have a small amplitude. Still possible, however, are enhanced, regional or seasonal responses in areas of extended land mass and lower thermal inertia. Currie[5] has found statistically significant evidence for such a variation of annual-averaged surface temperature in 53 stations in the northeastern sector of North America, based on recent analyses of 80 yr of surface data. The varation found by Currie has a mean amplitude of about 0.18 °C; it was absent or undetectable in other regions of North America for which extended records exist. The mean period of the temperature variation was 10.5–10.7 yr, which may be compared with the mean sunspot blocking period shown in Fig. 4 of ~10.6 yr. Currie found that the air temperature lagged sunspot numbers by 5.7±0.7 yr in the sense that temperatures tended to be lower at maxima in the 11-yr sunspot cycle; this is almost precisely what would be expected were the temperature effect due to sunspot blocking, with a reduced solar constant at times of high sunspot numbers. The apparent confirmation of a suspected regional, solar–climate effect is intriguing; before it can be accepted, however, it should be tested on other continents and with realistic climate models. But a small solar constant signal, restricted to an inland region geographically removed from the thermal inertia of the oceans and downwind of a major mountain chain is not unreasonable.

We have found further possible evidence in the same geographical region by examining the occurrence of temperature extrema in a data set[38] of mean monthly temperatures for the 48 contiguous US states over the time span 1900–80. The occurrence of a monthly high temperature record was a factor of 3 more frequent during the three years of minimal sunspot blocking than during the three years of maximum sunspot blocking. The same effect is found in the remaining regions of the continental US although with a slightly weaker signal. This factor of three greater probability of finding temperature maxima at minimum solar activity is a statistically significant result, with only a 1% probability of occurring by chance. The corresponding occurrence of record low temperatures during maximum sunspot blocking has a much weaker mirror relationship: record low temperatures were 30% more frequent in maximum than in minimum sunspot blocking years, although there is a 20% probability of this having occurred by chance.

Theoretically expected temperature response

What global surface temperature response should we expect from the reconstructed solar constant forcing? A reasonable answer is shown in Fig. 4, from a simple global climate model that assumes energy balance between incoming solar radiation and outgoing IR[39,40]. The model includes terms for the thermal inertia of the oceans: a mixing layer (upper level equivalent 87 m of water) with a thermal response scale of 5–10 yr, and a deep ocean with a volume 20 times larger. The effect of a

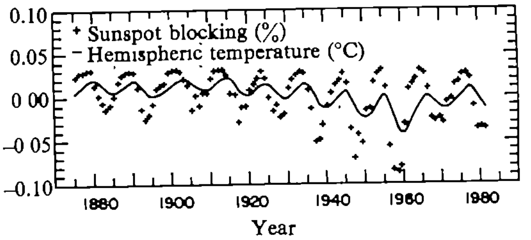

Fig. 4 The yearly mean deviations in solar irradiance indicated by the sunspot blocking model for 1874–1981 with expected hemispheric temperature variations as calculated using a simple energy balance climate model.

short-term change in insolation will be moderated by the large thermal inertia of the strongly-interacting surface regions (atmosphere, land surface and ocean mixed layer) of the Earth. The degree of attenuation will depend on the frequency and persistence of the forcing signal. A cyclic perturbation in insolation with a period of 11 yr will induce a surface temperature response that is a factor of 2–3 smaller than the equilibrium response to the same forcing. The equilibrium response of surface temperature to solar constant changes is generally estimated at 0.1 °C for a 0.1% radiative perturbation[41]. Peak-to-peak variations in solar forcing of 0.12% on an 11-yr cycle derived from extrapolated sunspot blocking models will lead to a similar cycle in mean global temperature with an amplitude of only 0.06 °C. Possible feedback mechanisms make this estimate uncertain by a factor of at least 2, but more detailed calculations of atmospheric circulation tend to support the energy balance arguments given above.

The atmosphere itself has a thermal inertia time scale far shorter than 1 yr (as evidenced by diurnal and seasonal variations). Therefore an inland region that is isolated from ocean temperature moderation can react to solar forcing in a manner more nearly that of the theoretical equilibrium response. Regions at high latitude will also experience larger responses due to the positive feedback of snow-albedo in which decreased solar radiation leads to greater snow cover, hence higher albedo and an even lower surface temperature. Currie's finding[5] of a weak, 11-yr cycle in inland North American air temperatures is therefore consistent in period, phase and amplitude with that expected theoretically from reconstructed sunspot blocking[14].

One can also test the compiled record of annual mean Northern Hemisphere temperature[42] for evidence of the same effect. We have done so and find that the mean hemispheric temperature during 1881–1981 was on average 0.036 °C cooler in the 3 yr of peak sunspot blocking as compared with the adjacent 3 yr of minimum solar activity. The reconstructed solar constant for the same period exhibits mean peak-to-peak variations of 0.06%; hence the observed temperature change is almost exactly what one should expect for a hemispheric average, based on simple climate models. Unfortunately, as is so often the case in solar–climate studies, the finding just cited is not statistically significant—about 50% of the time, given the statistics of the population sample, the same correspondence could occur from mere chance.

The response of surface temperature to forcing by sunspot blocking can be summarized as follows:

(1) On a hemispheric or global scale surface temperature responds to the 11-yr sunspot cycle, with an amplitude of 0.036 °C. However, this variation, although in accord with predictions of simple climate models, is not statistically significant.

(2) Station records for the past 80 yr in the north-east sector of the US establish that the mean surface temperature responds to solar, 11-yr forcing with an amplitude of a few tenths of 1 °C.

(3) A significant variation in the temporal distribution of record high monthly mean temperatures occurs in the same geographical region cited above, in response to solar forcing. The number of record low monthly mean temperatures also responds in a manner consistent with solar forcing, but not in a statistically significant way.

The phase of the relationship between sunspots and the solar constant, described above, is precisely opposite that which has been proposed to link longer-term changes in the envelope of solar activity with century-scale changes in climate[43]. The possible association of the Maunder and Sporer minima of solar activity, lasting 70 and 130 yr, with coincident cold periods of the Little Ice Age has been interpreted, for example, as the result of a postulated drop of about 1% in the solar constant[44]. The simple extrapolation of the mechanism of sunspot blocking to century-scale changes in the envelope of the activity cycle would induce an apparent secular modulation of at most 0.1%, with an increased solar constant at times of suppressed activity, such as the Maunder Minimum. Whether such an extrapolation is justified is another question. It is altogether possible that solar processes other than accumulated sunspot blocking act to modulate the solar constant in the longer term, and that such processes could oppose, in sense, the higher frequency modulation due to the 11-yr sunspot cycle. Such long-term changes are, of course, wholly speculative, since precision measurements of the solar constant span no more than 4 yr.

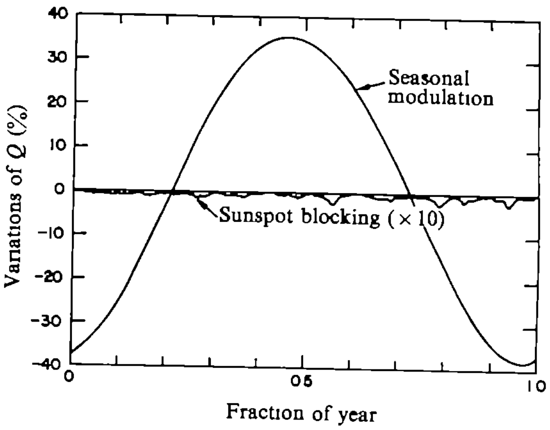

Fig. 5 Variation of daily solar irradiance (represented by Q) about the yearly mean due to seasonal effects in per cent deviation from a hemispheric average. The solar constant reconstruction for 1981 is shown multiplied by a factor of 10 for comparison.

Importance of climatic response

As shown above, both observation and theory seem to support the case for a small but direct surface air temperature response to measured and extrapolated solar constant fluctuations that is within a factor of 2 of the expected change in the solar luminosity.

These conclusions suggest several practical questions: (1) Is the apparent temperature response detectable as a perceptible weather effect? That is, can the average person sense a solar constant perturbation? (2) Will the anticipated climatic change be significant in practical or economic terms? (3) Can identifying the cause of climatic change at this level lead to better weather prediction?

Considering that the amplitude of the solar-induced mean temperature signal is but a few tenths of 1 °C, the answer to all of the above questions must surely be negative. A surface temperature effect of this magnitude is truly insignificant and for practical purposes imperceptible in the presence of typical diurnal extremes of 10° and a seasonal range of at least 30°. It is hard to see how a cyclic variation of a few tenths of a degree in the mean could have either predictive or economic import.

However, a factor of 3 enhancement in the frequency of temperature maxima occurring over a sunspot cycle could yield a marginally positive answer to all three of our pragmatic questions. People are much more likely to notice and remember unusual climate extremes. In economic terms it is again the extremes which cause dislocations. A factor of 3 change in the probability of occurrence of extrema in temperature is also of possible though limited predictive power.

Another way of gauging the climatic importance of a small perturbation to the solar constant is to compare it with the seasonal cycle of insolation. In Fig. 5 we compare the intra-annual variation of daily sunlight received over the Northern Hemisphere with the variation of S as reconstructed from sunspot blocking for 1981 (the latter is multiplied by 10 to make it visible). As one can see the interannual variation due to seasonal effects easily overwhelms that due to sunspot blocking. As another test, if we define the onset of spring to be 21 March in a year with 'normal' solar irradiance, how much will anticipated insolation changes due to sunspot blocking shift the effective first day of spring? In a year with high sunspot blocking (such as 1981 or 1982) the integrated irradiation reaches that of a normal spring about 0.2 days late. One effect of a sunspot-blocked solar constant could thus be the delay of the onset of spring by a few hours. By comparison, the meteorologically defined onset of spring, the date of last severe frost, has a typical variation of about 21 days and extreme differences of about 90 days in mid-latitude regions. Hence any shift due to the solar constant sunspot blocking is unimportant compared with typical variations, just as any induced change of mean surface temperature is also small compared with typical variations. This could be qualified, perhaps, by the fact that the mean sunspot blocking effect extends over a few years, thus allowing seasonal variations to be averaged over, making it slightly easier to detect the small effect.

Discussion

Solar constant variations of at most 0.5% for a few days and ~0.1% for a few years are now known to exist, but their expected effects on surface temperature are by any measure minor. Modelled or measured climate responses are insignificant compared with normally occurring changes and to apparently random variations in temperature. Thus it seems wholly unjustified to attribute the coincidence of a harsh winter such as that of the past year in some regions to a direct effect of a coincident solar constant deficit[28].

On the other hand, the importance of climate responses which might result from solar constant variations should not be dismissed. The identification of a simple, external forcing function such as a variable solar constant, which induces detectable changes of mean temperature, however slight, may provide a practical test of climate sensitivity on regional scales. Climate sensitivity verification is crucial to understanding such problems as the impact of anthropogenic carbon dioxide increases and land use changes. In a world where the demand for food is ever closer to the available supply, it may be true that any predictable fluctuation in climate will in time become important.

Perhaps the most important conclusion of the variations now established in the solar constant is that the Sun is indeed variable in its most fundamental output and that plausible responses of surface temperature have now been at least tentatively identified. Thus to increase our understanding of solar variability, we must continue the precision monitoring of the outputs of the Sun.

The demonstrated connection between changes in the solar constant and surface temperature exemplifies the kind of effort needed to redirect the study of solar–terrestrial relationships. Precision, spaceborne measurements of the solar output have enabled us to identify, and delimit, at least on time scales of days to years, a real climatic effect. Important questions remain unanswered. What are the detectable impacts of measured changes in solar short-wave radiation, or in the variable, particle inputs to the upper atmosphere? Do 'trigger' mechanisms have a role in amplifying the effects of variations in S or in other, weaker outputs of the Sun? Is the solar constant variable, perhaps in different or opposing ways, on longer time scales? New attempts at answering these and other such questions must surely focus not on statistical correlations but on mechanisms, modelling, and above all, measurements. Until we know how and in what ways the Sun varies we can only guess the possible terrestrial consequences.

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ARTICLES

The 5-min oscillations of the Sun are incompatible with a rapidly-rotating core

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Hill's suggestion that Einstein's general relativity may be invalid requires a rapidly spinning solar core. The triplet structure observed in the solar 5-minute power spectrum seems incompatible with this rotating core. But the triplet structure can be accounted for as the effect of a magnetically distorted core rotating with a ~12.5-day period.

FROM a study of the fine structure of solar oscillations Hill[1,2] has concluded that the Sun's core is rotating with a period of ~4 days. From a consideration of the effect of the resulting J_2 on Mercury's motion he concludes that Einstein's general relativity may be invalid[1]. From the same data Gough[3] agrees that the Sun contains a rapidly rotating core, but he disagrees with the implication *vis-à-vis* Einstein's theory.

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An even shorter rotational period (1 to ~2 days) was previously obtained from solar oblateness measurements (a P_2 distortion) made in 1966[4,5]. It was later found that a substantially better fit to the data could be obtained from an alternative hypothesis, that the solar distortion is not axially symmetric and that the distorted solar figure (the gravitational potential surface) rotates rigidly over the solar surface, as a wave, with a 12.38±0.10-day period (sidereal)[6,7]. It has been suggested that the 12.38-day period represents the rotational period of the core and that the distorted gravitational potential is due to a strong magnetic field buried in the solar core[7–9].

Clearly the 4-day period of Hill *et al.* and Gough, and this 12.4-day core rotation period are incompatible. Which if either is correct? The triplet structure discovered by Claverie, *et al.*[10] in the $l = 1$ '5-min' oscillations may yield the answer (see Fig. 1).

This triplet structure was first interpreted as rotational splitting of the $m = 1, 0, -1$ components of the $l = 1$ modes, the effect of advection of the modes[10]. With this interpretation the splitting is too small to be compatible with the ~4-day period and with the rotational curves given by Gough[3]. It yields a core rotation period of ~12 days for a rigidly rotating core with a radius of 0.6.

Isaak[11] has noted that owing to the symmetry of the observation, an average over the solar disk, the central ($m = 0$) component of the triplet (Fig. 1) should be missing for a solar axis perpendicular to the line-of-sight. For the actual solar axis tilt ~6° the $m = 0$ component should be very weak, with an intensity of 0.005. Isaak has suggested that the axial symmetry of the sun is broken by an off-axis magnetic field in the solar core rotating with a 12-day period and that the '$m = 0$' component is present because it contains an admixture of $|m| = 1$ induced by the magnetic field.

Gough[12] has noted that the same symmetry requirement would yield a triplet structure for rotational splitting of the $l = 2$ oscillations (without $m = \pm 1$ components), but he considers an $l = 2$ origin of the triplet implausible. With an $l = 2$ origin and the splittings given above (Fig. 1), the core of the Sun must rotate slower than the surface. This is very unlikely. Furthermore, Gough[12] considers the $l = 1$ identification to be statistically robust.

The fine structure of the $l = 1$ '5-min' oscillation seems to be incompatible with a purely rotational splitting induced by a rapidly rotating core. Is it compatible with the type of splitting to be expected from a magnetized solar core rotating rigidly with a 12-day period? Gough[12] has already examined this question making several simplifying assumptions and found that he could not obtain a satisfactory account of the splitting using this solar model.

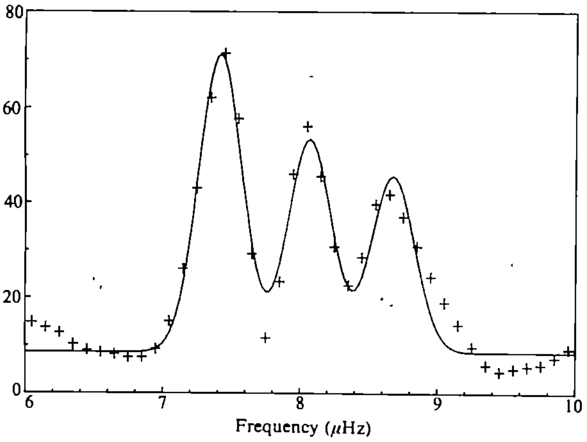

Fig. 1 Fine structure of $l = 1$ modes of '5-min' oscillations. The data points were taken from Fig. 2*b* in ref. 10. (The frequency origin is arbitrarily chosen.) The curve is an eight-parameter fit of the sum of a constant and three gaussians having a common width. The three frequencies obtained are: $\nu_1 = 7.419 \pm 0.009$, $\nu_2 = 8.071 \pm 0.012$ and $\nu_3 = 8.676 \pm 0.015$ μHz. The two frequency splittings differ by only 1.6σ. The mean splitting is 0.6285± 0.0087 μHz. The errors are only formal standard deviations. The gaussian width (σ) is 0.158 μHz.

Table 1 Matrices $R(\alpha)$, $P(\beta)$ and T

$\begin{bmatrix} \cos\alpha & -\sin\alpha & 0 \\ \sin\alpha & \cos\alpha & 0 \\ 0 & 0 & 1 \end{bmatrix}$	$\begin{bmatrix} \cos\beta & 0 & \sin\beta \\ 0 & 1 & 0 \\ -\sin\beta & 0 & \cos\beta \end{bmatrix}$	$\begin{bmatrix} & -1 & i & 0 \\ \frac{1}{\sqrt{2}} & 0 & 0 & \sqrt{2} \\ & 1 & i & 0 \end{bmatrix}$

Elements of the rotation matrices $R(\alpha)$ (z axis), $P(\beta)$ (y axis) and the transformation matrix T (for change of basis from W_n to $Y_{1,m}$).

One of Gough's assumptions can be questioned. He correctly notes that both the perturbation of the wave by the magnetically distorted core and the rotationally induced advection of the propagating wave are small effects and can be linearily superposed. He consequently assumes that the normal mode structure can be calculated by first neglecting the effect of solar rotation (including only the magnetically induced perturbation) and then adding the advection to this normal mode.

It is shown here that this assumption seems to be untenable. When the solar core is rotating more rapidly than the surface it is necessary to include the effect of advection before the normal oscillatory modes are calculated. Both the structure of the normal modes and their frequency perturbations can be strongly affected by advection.

Normal oscillatory modes

The normal modes for $l = 1$ are to be calculated assuming that the solar core (of radius r_c) is distorted by an internal magnetic field and is rotating rigidly with an angular velocity Ω_c. It is assumed that outside the core ($r_c < r < r_0$) $\Omega(r)$ is a function of r only. To avoid the complications arising in the time dependence of the magnetic perturbation we follow Gough's technique of employing a coordinate frame fixed to the core. The small Coriolis effect is neglected.

In this coordinate frame the solar core is static whereas the outer shell is rotating in a steady state. In the absence of dissipation the wave equation is time-reflection invariant in the core.

Expressed in terms of the radial displacement of the fluid ξ, the $l = 1$ unperturbed normal modes (complex) are conveniently separated into ingoing and outgoing waves, ξ_- and ξ_+

$$\xi_- = \sum_n a_n W_n(\theta, \phi) f(r) \exp(-i\omega t) \qquad (1)$$

$$\xi_+ = \sum_n b_n W_n(\theta, \phi) f^*(r) \exp(-i\omega t) \qquad (2)$$

(By an unperturbed normal mode is meant a normal oscillatory mode for a non-rotating Sun with an undistorted solar core.)

Near the centre of the Sun the complex radial function $f(r)$ has the form of the derivative of a spherical Hankel function

$$f(r) = -i \frac{d^2}{dr^2} (\exp(-ikr)/k^3 r) \qquad (3)$$

Elsewhere it depends on details of the solar model. $f(r) + f^*(r)$ is singularity free at $r = 0$. For the unperturbed normal mode the constant $b_n = a_n$. For the perturbed wave the (complex) a_n and b_n are functions of r.

The waves are expanded in real surface harmonics of $l = 1$ (W_n) which are in turn expressed in terms of the usual (normalized) complex spherical harmonics $Y_{l,m} \sim P_l^{|m|} e^{im\phi}$

$$W_1 = \frac{1}{\sqrt{2}} (-Y_{1,1} + Y_{1,-1})$$

$$W_2 = \frac{i}{\sqrt{2}} (Y_{1,1} + Y_{1,-1}) \qquad (4)$$

$$W_3 = Y_{1,0}$$

The real surface harmonics, W_n, are required to incorporate conveniently the time reflection invariance of the solar core.

The calculation of the three normal modes for $l=1$ and a fixed radial order is carried out in four steps:

(1) We start with the ingoing wave evaluated at the surface of the core and characterized by the three wave amplitudes a_1, a_2, a_3. The ingoing wave passes through the core and reappears at the surface as an outgoing wave characterized by b_1, b_2, b_3. Owing to the influence of the core magnetic field, the velocity of sound is not uniform on spherical surfaces and the wave propagating through the core undergoes an angle dependent phase shift. The outgoing amplitudes b_1, b_2, b_3 describe the $l=1$ part of this phase shift. The as and bs are linearily related. In matrix notation

$$b=Sa \tag{5}$$

where a and b are column vectors containing their three elements of the order (1)...(3). The so-called scattering matrix S is both unitary and symmetric. In the absence of the core distortion $S=I$ is the identity matrix.

(2) After leaving the core, the outgoing wave passes through the outer shell, is reflected at the solar surface and returns as an ingoing wave to the surface of the core where it has an amplitude a'. In this transit through the rotating shell the wave is rotated through a (negative) angle $\Delta\phi$ given by

$$\Delta\phi = 2\int_{r_0}^{r_0} (\Omega(r)-\Omega_c)\,\mathrm{d}r/c \tag{6}$$

where c is the sound velocity.

$$a' = R(\Delta\phi)\cdot b \tag{7}$$

where $R(\Delta\phi)$ is the rotation matrix which rotates b by $\Delta\phi$ into the orientation of a' (see Table 1).

(3) To represent a normal mode (a') must be identical with (a) except for the phase shift factor $\lambda = \exp(-i\Delta\omega\tau)$ due to shift $\Delta\omega$ in the angular frequency. Here $\tau \cong 7{,}400$ s is the round trip transit time of the wave. Combining equations (5) and (7) and setting $a' = \lambda a$ yields the eigenvalue equation

$$(RS)a_i = \lambda_i a_i \tag{8}$$

The matrix (RS) is unitary and its three eigenvalues λ_i are unimodular. For each of these eigenvalues there is an eigenvector a_v containing three components, representing one of the normal modes.

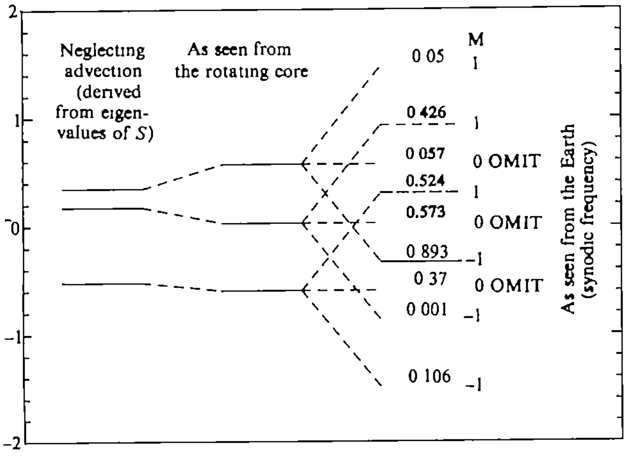
Frequency (μHz)
2
1
0
−1
−2
Neglecting advection (derived from eigen-values of S)
As seen from the rotating core
M
0 05
1
0 426
1
0 057
0 OMIT
0.524
1
0.573
0 OMIT
0 893
−1
0 37
0 OMIT
0 001
−1
0 106
−1
As seen from the Earth (synodic frequency)

Fig. 2 The computed fine structure of the $l=1$ component of the '5-min' oscillation of the Sun. The three phase angles of the scattering matrix, s_1, s_2, $s_3 = -0.0162$, -0.0081 and $+0.0242$, have ratios that differ only slightly ($\sim 1\,\sigma$) from the ratios of the three principal moments of inertia of the solar core. The mode advection angle is $\Delta\phi = -0.0167$ and the core orientation angles are $\alpha = 1.59$, $\beta = 0.787$ rad. The plotted frequencies are: (1) $\Delta\nu_i = -s_i/2\pi\tau$, for which the effect of advection is neglected ($\tau = 7{,}400$ s); (2) $\Delta\nu_i = \Delta\omega_i/2\pi$, equation (8), where the effect of advection $\Delta\phi$ is included but the mode is observed from the rotating core; (3) the same as (2) above but as viewed from the Earth (synodic frequencies) $\Delta\nu_{im} = (\Delta\omega_i + m\Omega_c)/2\pi$ with the spherical harmonic orders m and the line intensities labelled. The intensities are chosen to sum to unity for each mode. $\Omega_c/2\pi = 1/12.81\ \mathrm{day}^{-1} = 0.903\ \mu$Hz.

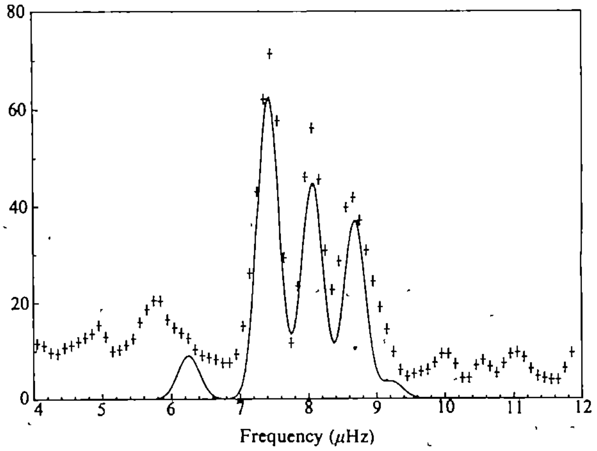

Fig. 3 Plot of the six spectral lines shown at the right of Fig. 2 against the data. The widths of the (guassian) lines are given by $\sigma = 0.158\ \mu$Hz.

(4) The above three values of $\Delta\omega$ would be observed in a coordinate frame rotating with the solar core. The transformation to an inertial coordinate frame is carried out by again using the rotational operator R. In inertial coordinates (primed) the ingoing wave amplitude (a_i) becomes

$$a_i' = R(\Omega_c t)a_i \tag{9}$$

This splits each normal mode (i) into three components with angular frequencies

$$\omega + \Delta\omega_i + m\Omega_c \tag{10}$$

of different order (m) spherical harmonics, $m = 1, 0, -1$. This is a purely kinematic effect (a rotational Doppler effect). In inertial coordinates the expansion (1) (2) in real harmonics is no longer convenient. Setting

$$\sum a_n W_n = \sum c_m Y_{1,m} \tag{11}$$

the spherical harmonic expansion coefficients c_m are obtained from the matrix equation

$$c = Ta \tag{12}$$

(see Table 1). Here the elements of c are of the order of $m = 1, 0, -1$. As seen from the Earth the (synodic) angular frequency is expression (10) with Ω_c replaced by $\Omega_c - 2\pi/365.25$ (day^{-1}).

The expression (10) divided by 2π yields nine different frequencies, three values of $m = 1, 0, -1$ for each normal mode $i = 1, 2$ and 3. But, as discussed above, the three $m = 0$ terms should be too weak to be observed. Depending on the scattering matrix S and the wave advection angle $\Delta\phi$ in expression (6) the remaining six frequencies can be of different intensities, some being so weak as to be unobserved.

The above discussed origin of the fine structure of the $l=1$ '5-min' normal modes is shown in Fig. 2 using the numerical example discussed below.

The scattering matrix

Evaluated at the surface of the core the intensity of the ingoing wave is proportional to $a\dagger a$, where $a\dagger = \tilde{a}^*$ is the Hermitian conjugate of (a). The outgoing wave intensity is $b\dagger b$ and for a lossless core $b\dagger b = a\dagger S\dagger Sa = a\dagger a$. This implies that $S\dagger = S^{-1}$, namely that S is unitary.

Because of time reversal invariance in the solar core, for any solution to the wave equation, $\xi(t)$, there is another $\xi^*(-t)$ and for the solution (a, b) there is another (b^*, a^*). Thus, $a^* = Sb^*$, $b = (S^*)^{-1}a$, $S = \tilde{S}$ and S is symmetric. Owing to these two properties of S, the matrix $i(S+1)(S-1)^{-1}$ is real. Thus its eigenvectors (and the eigenvectors of S) can be real and S can be diagonalized by an orthogonal matrix.

In the absence of a distortion of the core $S = I$ and for a first-order perturbation by a small core distortion, the correction to S is purely imaginary. This first-order correction to the outgoing wave amplitude (b) is calculated using Green's theorem, neglecting buoyancy effects.

$$\int [\eta \nabla_r \xi_1 - \xi_1 \nabla_r \eta] \rho c_0^2 r_c^2 \, d\Omega = \int_0^{r_c} \nabla \eta \cdot \nabla \xi_0 C \rho c_0^2 r^2 \, dr \, d\Omega \quad (13)$$

Here the left side is an integral over the surface of the core, ξ_1 is the first-order perturbation of the radial displacement, $c_0^2(r)$ is the unperturbed sound velocity squared, and $\eta = (f + f^*) W_n \exp(i\omega t)$.

The right side of equation (13) is an integral over the interior of the solar core, ξ_0 is the unperturbed radial displacement and the sound velocity (squared) is $c^2 = c_0^2(r)[1 + C(r, \theta, \phi)]$. The left side yields the perturbation of b_n and consequently the first-order correction to S. Only the $l = 2$ part of C contributes to the right side.

Any real second-degree spherical harmonic ($l = 2$) defines one or more sets of principal axes and can be expressed as a superposition of three second-degree Legendre polynomials, $P_2(n) = \frac{1}{2}(3 \cos^2 \theta_n - 1)$, referred to these three principal axes. The $l = 2$ part of the sound velocity anisotropy $C(r, \theta, \phi)$ can be expressed as

$$C_2(r, \theta, \phi) = h(r) \sum_{n=1}^{3} c_n P_2(n) \quad (14)$$

where $\sum_n c_n = 0$.

The right side of equation (13) is easily evaluated with the core first oriented to bring its principal axes into coincidence with the three coordinate axes. Equation (13) then yields a diagonal first-order perturbation of S with elements is_n. The diagonal matrix is designated as S_d, and has the eigenvalues $S_n = \exp(is_n)$ on its diagonal with $s_1 + s_2 + s_3 = 0$.

Substituting equation (14) in (13) and using the WKB approximation gives

$$s_n = -\frac{4\omega c_n}{5} \int_0^{r_c} h(r) \frac{dr}{c_0(r)} \quad (15)$$

To obtain the scattering matrix for the core in its proper orientation, the core is first rotated about the z axis through an angle α, then about the y axis through an angle β and finally again about the z axis. The last rotation can be omitted because it is equivalent to a rotation of the arbitrarily oriented coordinate system about the z axis. The scattering matrix of the reoriented solar core is

$$S = P(\beta) R(\alpha) S_d \tilde{R}(\alpha) \tilde{P}(\beta) \quad (16)$$

(see Table 1 for $P(\beta)$). As noted above, S can be diagonalized by an orthogonal matrix, in this case the matrix (PR).

A magnetic field in the solar core perturbs the velocity of sound in several different ways, primarily by perturbing the fluid density and pressure, and by the magnetic pressure. For a magnetic field which is primarily toroidal and derived from a spherical harmonic of definite degree (l), all of these contributions to the function $C(r, \theta, \phi)$ have the same angular dependence[9]. This angular dependence can be obtained from the principal moments of inertia of the core which are in turn obtained from the $l = 2$ part of the distortion of the solar surface[7].

Under a hypothesis to be discussed elsewhere the $l = 2$ part of the surface distortion can be calculated and yields -1.33 ± 0.23, -0.36 ± 0.22 and 1.69 ± 0.15 ($\times 10^{50}$ g cm^2) for the three (traceless) principal moments of inertia of the solar core. (The significance of these results for the nutation and the oscillation of the core is to be discussed elsewhere.) If the above assumptions are valid the three eigenvalue phase angles s_i should be proportional to the above three traceless moments of inertia (I_i) namely

$$s_i / s_j = I_i / I_j$$

and

$$s_i = K I_i \quad (17)$$

Numerical results

Is the fine structure splitting seen in the series of $l = 1$ '5-min' oscillations compatible with a magnetically distorted core rotating with a 12.38-day period (sidereal) as derived from the 1966 observations[5] of the 'solar oblateness'? Owing to the nutation and a possible long-period oscillation[9] expected with a magnetically-distorted core, the core orientation obtained from the 1966 data is not expected to be valid in 1981, but the eigenvalue phase angle ratios in expression (17) are assumed to be applicable with a 1 σ accuracy. With these restrictions the multiplier K in equation (17) and the angles α, β and $\Delta\phi$ are assumed to be freely adjustable.

By adjusting these four parameters the following conditions should be met: (1) the intensities of the six frequencies with $m = \pm 1$ should divide into three intense spectral lines and three negligible weak ones; (2) the intensity ratios of the strong lines should be acceptable; and (3) their frequency differences should be correct. The example illustrated in Fig. 2 with the parameters given in the caption is considered to be satisfactory. There is no *a priori* reason for the three normal modes to be equally excited and the indicated intensities (assuming a total intensity of 1 for each normal mode) are satisfactory.

In Fig. 3 the six spectral lines shown in Fig. 2 are plotted with the data after adjusting the total intensities of the three normal modes to be in the ratios of 0.985/1.000/0.807 in order of increasing frequency $\Delta\omega_i / 2\pi$.

Conclusions

With the assumption that the solar rotation outside the core is substantially uniform, the advection (rotation) angle $\Delta\phi = -0.0166$ permits a calculation of the radius of the core using equation (6). The core is found to have a radius half that of the Sun (3.5×10^{10} cm).

Assuming a distorted mass shell of this radius and the principal moments of inertia given above yields a $\delta\rho/\rho$ contribution to the values of $s_1 \ldots s_3$ with magnitudes roughly 1.8 times the above, but with opposite sign. Owing to the important role of the magnetic pressure and the distorted gas pressure, which contribute with signs opposite to $\delta\rho/\rho$, it is impossible to obtain better estimates for s_i without knowing more about the distribution of the magnetic field.

Apparently a magnetically distorted solar core rotating with

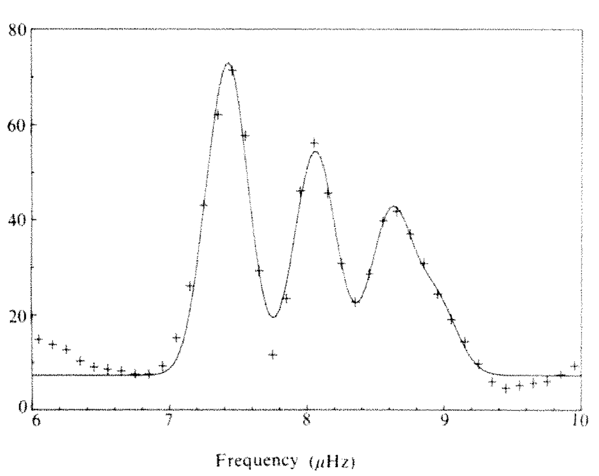

Fig. 4 Same as Fig. 1 but including four gaussians in the least-square-fit to the data points. In order of increasing frequency the intensity and frequency of each of the three spectral lines are 65.7 ± 2.0, 7.420 ± 0.006 μHz; 47.2 ± 2.0, 8.063 ± 0.008; 33.9 ± 2.7, 8.613 ± 0.018 and the fourth weak peak of unknown cause are 15.1 ± 2.7, 8.941 ± 0.039. The difference between the two principal frequency separations is 0.093 ± 0.025 μHz. Thus the two frequency splittings are unequal at a 4σ level.

a 12.38-day period can give a satisfactory account of the triplet structure of the $l=1$ '5-min' oscillations. But there is one unsatisfactory feature in the above account. The three gaussians fitted to the data in Fig. 1 give equal line splittings for the triplet to within 2σ. But, from the standpoint of the above analysis this is a fortuitous result. We have no explanation for the equality.

But are the splittings between the three spectral lines actually equal? An examination of the high frequency component in Fig. 1 shows a poor fit to the data. There appears to be a blend with some unidentified weak fourth line, perhaps due to noise or aliasing. By adding a fourth gaussian a satisfactory fit to the high frequency component is obtained (see Fig. 4). Now the splittings of the three principal components are unequal at a statistically significant level (4σ).

For the above interpretation of the $l=1$ fine structure to be valid, the fine structure would be expected to vary significantly in a year or two, owing to nutation and/or torsional oscillation[9] of the core.

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Garnet–orthopyroxene barometry for granulites and peridotites

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An experimentally calibrated geobarometer based on the solubility of alumina in orthopyroxene coexisting with garnet is presented. This geobarometer is based on experiments in the $FeO–MgO–Al_2O_3–SiO_2$ *and* $CaO–FeO–MgO–Al_2O_3–SiO_2$ *systems performed at high temperatures and pressures (800–1,200 °C and 5–20 kbar). Applying the geobarometer to a variety of natural garnet–orthopyroxene assemblages from granulite facies terrains and other occurrences, yields reliable and consistent pressure estimates for these samples.*

PETROLOGISTS concerned with garnet peridotite xenoliths in kimberlitic diatremes have long recognized the importance of the solubility of alumina in orthopyroxene coexisting with garnet as a pressure (P)–temperature (T) indicator[1–4]. More recently, petrologists have applied the garnet–orthopyroxene geobarometer to estimate the physical conditions of equilibration of crustal granulites[5,6]. Earlier experimental calibrations of the variation in the alumina content of enstatite coexisting with pyrope as a function of P and T in the system $MgO–Al_2O_3–SiO_2$ (MAS) have been used widely, in conjunction with other geothermometers, to construct 'palaeogeotherms' for mantle-derived garnet peridotites[7–11]. More recent experimental data in the system $MgO–Al_2O_3–SiO_2$ (refs 12–16) have tended to confirm the $P–T$ dependence of alumina in enstatite coexisting with pyrope that was obtained by MacGregor[7]. Recent results in $CaO–MgO–Al_2O_3–SiO_2$ (CMAS)[13,17] have, however, necessitated a revision in pressures estimated from alumina contents of orthopyroxenes coexisting with pyrope–grossular garnets such as those which occur in garnet peridotites. Major problems encountered in the application of these experimental studies undertaken in simple end member systems to natural rocks, are the effect of the Fe^{2+} contents of the phases on the alumina solubility at any given $P–T$ condition, and the lack of adequate experimental data at $P–T$ conditions directly applicable to natural granulite assemblages. Previous experimental studies in Fe-bearing systems[5] have been restricted in the compositional range studied, and have yielded only unreversed data for the alumina contents in orthopyroxenes coexisting with a garnet phase. For these reasons, and because of the widespread occurrence of garnet–orthopyroxene assemblages in crustal granulites, we have experimentally recalibrated the garnet–orthopyroxene geobarometer/geothermometer in the $FeO–MgO–Al_2O_3–SiO_2$ (FMAS) and $CaO–FeO–MgO–Al_2O_3–SiO_2$ (CFMAS) systems, over a wide range in X_{Mg} (Mg/Mg+Fe) and X_{Ca}(Ca/Ca+Mg+Fe) bulk compositions at any one $P–T$ condition, and at $P–T$ conditions appropriate to the formation of crustal granulites (800–1150 °C and 5–20 kbar).

Thermodynamic treatment of garnet–orthopyroxene equilibria

The reaction buffering the alumina content of orthopyroxene coexisting with garnet may be written as[5,7,14]:

$$\begin{array}{ccccc} Mg_2Si_2O_6 & + & MgAl_2SiO_6 & = & Mg_3Al_2Si_3O_{12} \\ \text{Enstatite} & & \text{Mg-Tschermaks'} & & \text{Pyrope} \\ \text{component} & & \text{component} & & \text{in garnet} \\ & & \text{Orthopyroxene} & & \end{array} \qquad (1)$$

Reaction (1) above is useful for geobarometry because of the large volume change involved ($=200$ cal kbar^{-1} mol^{-1}).

The equilibrium condition for reaction (1) at a given pressure, P, and temperature, T, is:

$$\Delta H^\circ - T\Delta S^\circ = RT \ln (a^{op}_{En})(a^{op}_{MgTs})/(a^{ga}_{py}) - P\Delta V^\circ \qquad (2)$$

where ΔH° and ΔS° are, respectively, the enthalpy and entropy changes of reaction (1); ΔV° is the standard molar volume change of reaction (1) at 298K; and the as represent the activities of the components in orthopyroxene and garnet.

The aim of our experimental approach is to extract values of $\Delta H^\circ_{1,T}$ and ΔS°_T from compositional data obtained over a $P–T–X$ (composition) range. To do this we require an expression or value for ΔV°, and a knowledge or model of the activity–composition relatioships of the phases involved. While we recognize that full, explicit, thermodynamic models of the garnet and orthopyroxene solid solutions are desirable, and that site distribution data exist for orthopyroxene in some chemical systems[18,19], the nature of the data obtainable from

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Table 1 *P–T* estimates for garnet–orthopyroxene assemblages

Locality	Description	Ref.	T(°C)	P (equation (5)) (k bar)	P (other) (kbar)
Greenland	Early Archaean coronites	47	625–700*	9–11.5	>8.0
Scourie	Metabasites	6	775–835*	6–10.5	silli
Greenland	Late Archaean granulites	48	750, 720*	10, 6.5	7.5
Scourie	Metabasites, coronites	49	725–825*	8, 14.5	silli
Aldan, USSR	Gar granulites	32	925†	10.7–12	silli
Kola, USSR	opx + sill + gar granulites	30	960–1,020†	9–10.5	silli
West Australia	Archaean granulites	50	770†	3.5	silli
Vredefort	Coronas in pseudotachylite	51	700†	5.0	silli
Uganda	Sapphirine bearing granulite	33	1,000†	10	8.1
Nain	Granulites around anorthosite	38	670–859†	2–7	3.3
		39	750 av.	3–4	FaFsQz
Norway	Metadolerites, coronites	52	650*	10–12.5	
Adirondacks	Metadolerites, coronites	53	650*	7.3, 16	8.5, 750 °C
India	Charnockites, mafic granulite	54	800–850‡	7.5–9.5	silli
		55	700–750*	8.4–9.1	silli
		56	850‡	5.0–7.0	7.4
Lesotho	Granulite xenoliths in kimberlite	57	600–700*	11.5–17.5	
Australia	Xenoliths in diatremes	41	900*	14	
		42	925*	12.5	
		43	775*	14	
	Spinel-garnet lherzolites	45	1,200*	22–24	25.1 O'Neill
Arizona	Garnet pyroxenite xenolith	44	710*	15.5	
Enderby Land	Sapphirine-bearing granulites	36	950§	7.0–9.0	7–10
Stanovik, USSR	Aluminous granulites	31	830–875†	4.0–5.2	
			950†	6.5, 9.0	
Ronda, Spain	Recrystallized neoblasts in peridotite massif	58	1,050*	20–21	
			900‡	15–17.5	
Cima de Gagnone	Garnet peridotite body in crustal gneisses	59	900* core	35.0	
			700* rims	22–30	
Selje, Norway	Orthopyroxene eclogite boudin in gneiss	60	650*	18.1	
Lien, Almklovdalen, Norway	Garnet peridotite in ultramafic body in gneiss	61	800* core	32.0	
			750* rims	30.1	
		62	650*	20.2	
Scourie	Metaigneous granulites 275	63	840*	9.3	
	5		875*	11.0	840 °C, 10.7
	9		860*	8.2	gar-bi
	217		800*	7.0	gar-opx-
	62		780*	6.4	plag-qz
	196		745*	6.7	
	199		700*	5.0	

* Garnet–clinopyroxene thermometer[64]. † Garnet–orthopyroxene thermometer[65]. ‡ Two-pyroxene thermometer[34]. § Phase petrology constraints.

the experiments presented here precludes the use of complicated multi-parameter solution models. A simpler approach which allows direct application to natural rock data has been pursued. Simple solution models applicable in the MAS and CMAS systems have been adopted, and essentially empirical curve-fitting parameters which describe the compositional dependences of Al-solubility in orthopyroxene in the Fe-bearing systems have been added. By taking the solid solution properties of the phases into account, the empirical terms have a similar form to terms describing non-ideal solid solution behaviour in more rigorous formulations.

In the MAS system, the activity of garnet is unity. Using the two-site model of Wood[5] for orthopyroxene, and assuming that no Al enters the M2 site:

$$a^{\mathrm{op}}_{\mathrm{En}}a^{\mathrm{op}}_{\mathrm{MgTs}} = X^{\mathrm{M1}}_{\mathrm{Al}}(1-X^{\mathrm{M1}}_{\mathrm{Al}})\gamma^{\mathrm{M1}}_{\mathrm{Mg}}\gamma^{\mathrm{M1}}_{\mathrm{Al}}$$

Recent work indicates that some Al may enter the M2 site[19], however, the simpler model above is adopted to allow development of a practical geobarometer.

Following Wood and Banno[20], Mg–Al orthopyroxenes have been assumed to mix ideally at 1 bar, and non-ideal interactions at higher pressure have been arbitrarily ascribed to an excess volume of mixing term ΔV_{ex}. Substitution of this term, derived from available molar volume data[14,21], for the standard molar volume change of reaction (ΔV°), results in a *P–T*-dependent volume change of reaction, ΔV_{r}:

$$\Delta V_{\mathrm{r}} = -[183.3+178.98(X^{\mathrm{M1}}_{\mathrm{Al}}(1-X^{\mathrm{M1}}_{\mathrm{Al}}))] \quad \mathrm{cal\ kbar^{-1}} \qquad (3)$$

Thus, in MAS:

$$\Delta G^{\circ}_{1,\mathrm{T}} = RT\ln[X^{\mathrm{M1}}_{\mathrm{Al}}(1-X^{\mathrm{M1}}_{\mathrm{Al}})]-P\Delta V_{\mathrm{r}}$$

With the addition of Fe into the system, both garnet and orthopyroxene vary in composition with *P* and *T*. In orthopyroxene several effects will arise from non-ideal interactions occurring on either 'M1' or 'M2' site or across sites (reciprocal terms). While recognizing these complexities and that garnet is no longer a pure component, the effects of adding Fe to the MAS system have been ascribed to one, empirical, curve-fitting term which accounts for the variation of Al-solubility in orthopyroxene with $X^{\mathrm{op}}_{\mathrm{Fe}}$ at one *P–T* condition (Fig. 1). This excess term is dominated by non-ideal Fe–Al interactions in the M1 site of orthopyroxene and is of the form:

$$\Delta H_{\mathrm{ex}} = W^{\mathrm{M1}}_{\mathrm{FeAl}}(1-X^{\mathrm{M1}}_{\mathrm{Al}})(1-2X^{\mathrm{M1}}_{\mathrm{Al}})X^{\mathrm{op}}_{\mathrm{Fe}}$$

with

$$X^{\mathrm{op}}_{\mathrm{Fe}} = \mathrm{Fe/Mg+Fe\ in\ orthopyroxene\ and}$$

$$X^{\mathrm{M1}}_{\mathrm{Al}} = \mathrm{Al/2\ in\ 6\ oxygen\ unit\ orthopyroxene}$$

Note that the $W^{\mathrm{M1}}_{\mathrm{FeAl}}$ parameter derived here is an empirical term only, and its magnitude is the net result of a number of effects in the orthopyroxene solid solution. Nevertheless, the ΔH_{ex} term adequately describes the deviation of Al-contents of Fe–Mg orthopyroxenes coexisting with garnet from those of an Mg-orthopyroxene at the same *P–T* condition.

In the CFMAS system, further terms must be introduced to account for the decrease in alumina contents of orthopyroxenes

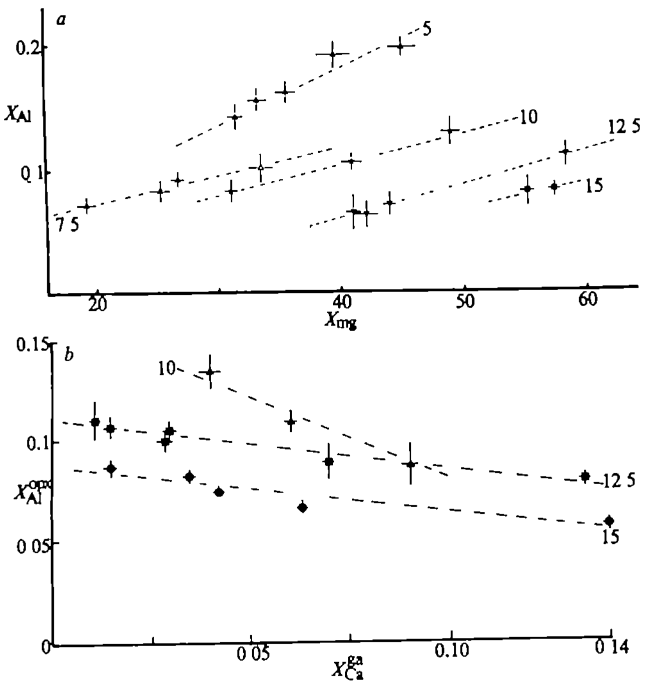

Fig. 1 *a*, Example of an experimental data set in the FMAS system showing the dependence of Al ($X_{\mathrm{Al}} = \mathrm{Al}/2$) solubility in orthopyroxene on Fe–Mg substitution in the pyroxene at 1,248K. Dashed lines join isobaric data, with pressures as indicated. Similar data has been obtained at 1,173K, 1,323K and 1,423K. $X_{\mathrm{Mg}} = (\mathrm{Mg}/\mathrm{Mg}+\mathrm{Fe}) \times 100$ in orthopyroxene. Error bars, one standard deviation (1σ) based on 10–15 orthopyroxene analyses. *b*, Example of an experimental data set in the CFMAS system showing the dependence of Al-solubility in orthopyroxene on the grossular content of coexisting garnet, at near-constant $X_{\mathrm{Mg}}(X_{\mathrm{Mg}}^{\mathrm{op}} = 60)$ and at 1,248K. Dashed lines join isobaric data, with pressures as indicated. Similar data has been obtained at 1,323K. $X_{\mathrm{Ca}}^{\mathrm{ga}} = (\mathrm{Ca}/\mathrm{Ca}+\mathrm{Mg}+\mathrm{Fe})$ in garnet.

with increasing Ca-contents of coexisting garnet. In comparison with the FMAS equation used above, where $X_{\mathrm{py}}^{\mathrm{ga}}$ is left as unity, in CFMAS the equation is adjusted using two terms:

(1) A dilution term incorporated into the expression for ln *K*

$$X_{\mathrm{py}}^{\mathrm{ga}\;3} = (1 - X_{\mathrm{Ca}}^{\mathrm{ga}})^3$$

(2) An additional term derived from a ternary regular-symmetric solid solution model for Ca–Mg–Fe garnets, with the constraints obtained from other phase equilibrium experiments that Ca–Fe and Fe–Mg interactions in garnet are near-ideal in the *P–T* range of interest[22–24]:

$$RT \ln \gamma_{\mathrm{py}}^{\mathrm{ga}} = W_{\mathrm{CaMg}}^{\mathrm{ga}} [X_{\mathrm{Ca}}^{\mathrm{ga}} X_{\mathrm{Fe}}^{\mathrm{ga}} + (X_{\mathrm{Ca}}^{\mathrm{ga}})^2]$$

where

$$X_{\mathrm{Ca}}^{\mathrm{ga}} = \mathrm{Ca}/\mathrm{Ca}+\mathrm{Mg}+\mathrm{Fe}, \text{ and } X_{\mathrm{Fe}}^{\mathrm{ga}} = \mathrm{Fe}/\mathrm{Ca}+\mathrm{Mg}+\mathrm{Fe}.$$

Note that this second term results in a derived $W_{\mathrm{CaMg}}^{\mathrm{ga}}$ which is a curve-fitting parameter only, as the term $(1 - X_{\mathrm{Ca}}^{\mathrm{ga}})$ rather than $X_{\mathrm{Mg}}^{\mathrm{ga}}$ has been used for the mole fraction of garnet. Even though the derived $W_{\mathrm{CaMg}}^{\mathrm{ga}}$ is an empirical parameter, the equations used here should give the correct form of Ca–Mg interaction in garnet.

Thus, in CFMAS an equation is derived which expresses the alumina content of orthopyroxene coexisting with garnet as a function of *P*, *T*, $X_{\mathrm{Fe}}^{\mathrm{op}}$, $X_{\mathrm{Ca}}^{\mathrm{ga}}$ and $X_{\mathrm{Fe}}^{\mathrm{ga}}$:

$$\begin{aligned} \Delta G^{\circ}_{1,T} = {} & RT \ln [X_{\mathrm{Al}}^{\mathrm{M1}} (1 - X_{\mathrm{Al}}^{\mathrm{M1}})/(1 - X_{\mathrm{Ca}}^{\mathrm{ga}})^3] - P\Delta V_{\mathrm{r}} \\ & + W_{\mathrm{FeAl}}^{\mathrm{M1}} (1 - X_{\mathrm{Al}}^{\mathrm{M1}})(1 - 2X_{\mathrm{Al}}^{\mathrm{M1}}) X_{\mathrm{Fe}}^{\mathrm{op}} \\ & + 3 W_{\mathrm{CaMg}}^{\mathrm{ga}} [X_{\mathrm{Ca}}^{\mathrm{ga}} X_{\mathrm{Fe}}^{\mathrm{ga}} + (X_{\mathrm{Ca}}^{\mathrm{ga}})^2] \end{aligned} \quad (4)$$

The development of this equation has essentially relied on the application of compositional correction factors, derived from either the orthopyroxene (in FMAS) or the garnet (in CFMAS) solid solutions, to a simple equilibrium relation applicable in MAS, and thus the derived parameters are partly empirical and may incorporate a greater number of more subtle effects in the solid solutions considered.

Experimental data on garnet–orthopyroxene equilibria

Equation (4) may be used in the reduction of experimental data obtained over a range of P–T–X_{Mg}(bulk)–X_{Ca}(bulk) conditions, given that values of $X_{\mathrm{Al}}^{\mathrm{M1}}$, $X_{\mathrm{Mg}}^{\mathrm{op}}$, $X_{\mathrm{Ca}}^{\mathrm{ga}}$ and $X_{\mathrm{Fe}}^{\mathrm{ga}}$ can be obtained experimentally. In this experimental study, two sets of experiments designed to obtain data sets for these compositional parameters have been undertaken:

(1) Experiments in the system FMAS at a range of X_{Mg}(bulk) values (=0.15, 0.30, 0.45, 0.50, 0.60, 0.70, 1.0), with bulk compositions appropriate to form garnet–orthopyroxene–quartz at a given *P–T* condition. These have been performed with both almandine and Al-free enstatite mineral mixes (X_{Mg}(bulk) = 0.33), and glass mixes seeded with 5% almandine or pyrope to promote garnet growth. It is considered that mineral-mix type starting materials constitute reversal mixes to glass starting materials, an assumption confirmed by analyses of orthopyroxenes grown from glass ($X_{\mathrm{Al}}^{\mathrm{M1}}$ decreases rimwards) and from enstatite seeds ($X_{\mathrm{Al}}^{\mathrm{M1}}$ increases rimwards).

(2) Experiments in CFMAS at X_{Mg}(bulk) = 0.50 and over a range of X_{Ca}(bulk) values (= 0.02, 0.04, 0.08) so as to produce garnets of differing $X_{\mathrm{Ca}}^{\mathrm{ga}}$ at essentially one P–T–$X_{\mathrm{Mg}}^{\mathrm{op}}$ condition. In these experiments, glass starting materials with 5% almandine seeds have been used exclusively, and the effect of $X_{\mathrm{Ca}}^{\mathrm{ga}}$ on $X_{\mathrm{Al}}^{\mathrm{M1}}$ has been evaluated by comparison of compositional data in this set of experiments with data previously obtained and reversed in FMAS.

All experiments have been performed in a Boyd-type, 1/2-inch, solid–medium, piston–cylinder apparatus. Temperatures were monitored by Pt/Pt90Rh10 thermocouples to within ± 10 °C of the set point during the prolonged run durations involved in ensuring subsolidus equilibration (4 days to 1 month, depending on the temperature). Pressures, monitored by Heise gauge, are believed to be accurate to within 0.5 kbar. All runs were of piston-in type, and a −10% friction correction has been applied to all nominal gauge pressures as talc outer sleeves were used. All samples were loaded into 3- or 4-bore Fe-metal or graphite capsules which were then sealed in noble metal outer capsules. Thus three or four mixes of differing bulk composition could be simultaneously run at one *P–T* condition.

Examples of the effects of $X_{\mathrm{Mg}}^{\mathrm{op}}$ and $X_{\mathrm{Ca}}^{\mathrm{ga}}$ on $X_{\mathrm{Al}}^{\mathrm{M1}}$, at various pressures for a given temperature, are illustrated in Fig. 1. The

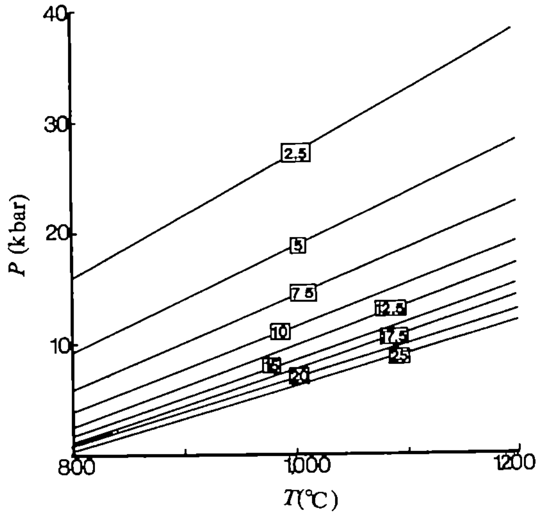

Fig. 2 X_{Al} (Al/2) isopleths for hypothetical orthopyroxene with $X_{\mathrm{Mg}}^{\mathrm{op}} = 50$, in equilibrium with Fe–Mg garnet. Isopleths are expressed as % Al in the M1 site.

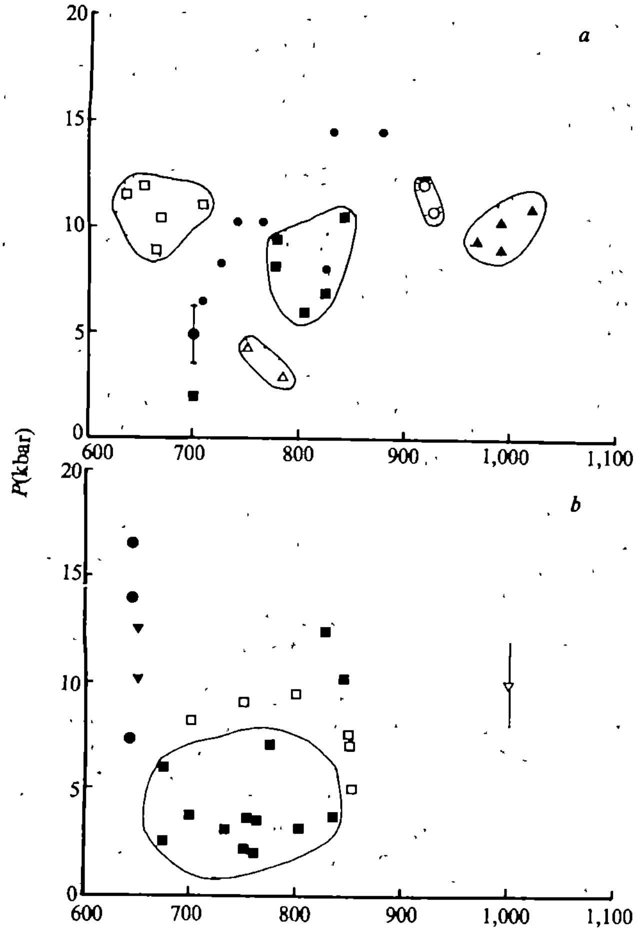

Fig. 3 P–T estimates for published examples of garnet–orthopyroxene-bearing granulites. P estimated from equation (5), T estimates as indicated in Table 1. *a*: □, Greenland[47]; ■, Scourie[6]; ○, Aldan Shield[32]; ▲ Kola Peninsula[30]; △, West Australia[50]; ●, Vredefort,[51]; •, Greenland and Scotland[48,49]. *b*, ■ Nain, Labrador,[38,39]; ▼, Norway[52]; ●, Adirondacks[53]; □, India[54–56]; ▽, Uganda[33].

principal effects to be noted are:

X^{M1}_{Al} decreases with increasing P at constant T, X^{op}_{Mg} and X^{ga}_{Ca}

X^{M1}_{Al} increases with increasing T at constant P, X^{op}_{Mg} and X^{ga}_{Ca}

X^{M1}_{Al} increases with increasing X^{op}_{Mg} at constant P, T and X^{ga}_{Ca}

X^{M1}_{Al} decreases with increasing X^{ga}_{Ca} at constant P, T and X^{op}_{Mg}

The experimental data in FMAS, a total of 75 data points, have been fitted to equation (4), with $X^{ga}_{Ca} = 0.0$, yielding best-fit multiple and stepwise regression parameters as follows:

$$\Delta H^{\circ}_{1,T} = -5{,}650 \pm (250)\ \text{cal mol}^{-1}$$

$$\Delta S^{\circ}_{T} = -2.93 \pm (0.30)\ \text{cal mol}^{-1}\ \text{K}^{-1}$$

$$W^{M1}_{FeAl} = 5{,}157 \pm (200)\ \text{cal mol}^{-1}$$

(figures in brackets are standard errors at the 1σ level).

Compositional data in CFMAS at $X^{op}_{Mg} = 0.60$ have then been fitted to equation (4) using the parameter values above, to obtain a least-square fit value of W^{ga}_{CaMg}

$$W^{ga}_{CaMg} = 2{,}100 \pm (200)\ \text{cal mol}^{-1}$$

Correction of equation (4) for excess molar volume terms in CaMg garnet[25], leads to a value of 2,530±200 cal mol^{-1} for this empirical parameter, in reasonable accord with values of W^{ga}_{CaMg} obtained in other studies[24,26,27].

Equation (4) may now be rearranged into an equation expressing P (kbar) in terms of T, X^{op}_{Mg}, X^{M1}_{Al}, X^{ga}_{Ca} and X^{ga}_{Fe}. This equation is useful as a geobarometer in granulite facies garnet–orthopyroxene assemblages and in garnet periodotites:

$$\begin{aligned} P\ (\text{kbar}) &= \frac{1}{\Delta V_r}[(R \ln K - 2.93)T + 5{,}650 \\ (\pm 1\ \text{kbar}) &\quad + 5{,}157(1 - X^{M1}_{Al})(1 - 2X^{M1}_{Al})X^{op}_{Fe} \\ &\quad - 6{,}300[X^{ga}_{Ca}X^{ga}_{Fe} + (X^{ga}_{Ca})^2] \end{aligned} \qquad (5)$$

where T is in (Kelvin; $K = [X^{M1}_{Al}(1 - X^{M1}_{Al})/(1 - X^{ga}_{Ca})^3]$; $\Delta V_r = -[183.3 + 178.98(X^{M1}_{Al}(1 - X^{M1}_{Al}))]$ cal kbar^{-1}.

The geobarometer will be limited by the following circumstances: (1) where Fe^{3+} contents are high in garnet or orthopyroxene, such that other non-ideal terms may be important in the garnet, or the $Fe^{3+}AlMg_{-1}Si_{-1}$ exchange may be important in orthopyroxene[28,29]; (2) where Cr^{3+} contents are high in garnet; (3) where Mn^{2+} contents are high in the phases.

In addition, the barometer will be subject to considerable uncertainty when either (1) Al_2O_3 contents in orthopyroxene are very low (such as high pressure garnet peridotites, or high Fe–Ca garnet assemblages formed at low temperatures), or (2) there are large uncertainties in the temperature estimate, as the dP/dT slopes of the Al_2O_3 isopleths are approximately 4 kbar per 100 °C (Fig. 2).

Applications of garnet–orthopyroxene geobarometry

The garnet–orthopyroxene geobarometer developed here (equation (5)) has been applied to several well-documented examples of granulite facies metamorphics, and to low-Cr garnet peridotite xenoliths in kimberlites from South Africa. The results of these calculations are presented in Fig. 3 and Table 1, together with the P–T conditions suggested by other workers for these samples on the basis of other geothermobarometric techniques or phase stability constraints.

Sillimanite- or sapphirine-bearing garnet–orthopyroxene assemblages reported from the USSR[30–32] and Uganda[33] yield P estimates which are consistent with the stability of sillimanite and with the stability of the assemblage orthopyroxene–sillimanite–quartz at 1,000 °C (ref. 34). Granulites from Enderby Land, Antarctica[35], yield pressures of 7–9 kbar, consistent with the stability of sillimanite in interlayered metapelites and with phase assemblages in associated aluminous granulites[36].

Retrograde garnet-bearing coronas in meta-igneous granulites from several areas (Table 1) yield pressure estimates in the range 7–12 kbar, which is consistent with the reported incoming of kyanite in metapelites associated with these coronites in some areas[37]. Unrealistic or highly uncertain P estimates are obtained, however, for some of these corona textures (for example, Adirondacks, Scourie). This may result from inadequacies in the experimental calibration at extreme Ca- and Fe-rich compositions, from the large errors inherent in the barometry at low X^{M1}_{Al} values, or from failure to obtain adjacent analyses of minerals in local equilibrium in otherwise zoned coronas. The Nain[38], Labrador, granulites, which occur in a thermal aureole around an anorthosite pluton, yield an average pressure estimate consistent with pressures of 3.2 kbar recently obtained for these rocks from olivine–orthopyroxene–quartz equilibria[39,40].

Granulitic and websteritic nodules from diatremes in southeastern Australia and the USA[41–44] yield pressures of 12.5–16 kbar, which are in good agreement with pressures inferred by Irving[42] for the equilibration of scapolite granulites from Delegate, New South Wales. Spinel–garnet lherzolite xenoliths from southeastern Australia[45], estimated to have equilibrated at 25 kbar by O'Neill[46] using the spinel to garnet peridotite transition as a barometer, yield pressures of 22–24 kbar using equation (5).

Comparison with most alternative pressure estimates considered here indicates that the experimentally derived geobarometer presented in equation (5) can be used successfully to estimate the pressures of formation and equilibration of garnet–orthopyroxene assemblages, provided a reliable temperature estimate can be obtained from other equilibria. Calculated pressures will, however, be heavily dependent on the deduced temperature. As in all geothermometry/geobarometry using several equilibria, it is also necessary to assume that closure temperatures are the same for the equilibria used.

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Relaxation of muscle fibres by photolysis of caged ATP

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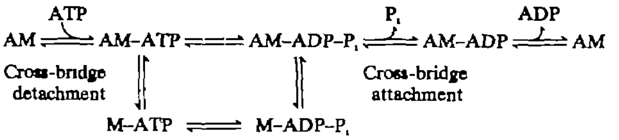
A novel method has been developed for studying the reaction kinetics of the force-generating mechanism in muscle. Inert photolabile precursors of ATP or ADP are incorporated into muscle fibres having their surface membrane barrier removed. The nucleotide is then rapidly liberated by laser pulse photolysis. This circumvents the limitation in time resolution set by diffusion of nucleotide from the medium bathing the fibre. This laser photolysis method may be applicable to studies of the dynamic properties of many biological systems.

THE energy transducing mechanism of muscle is widely thought to involve a cyclic reaction of cross-bridges between thick and thin filaments[1,2]. Lymn and Taylor proposed a scheme relating the chemistry of the actomyosin ATPase to muscle contraction[3]. This scheme, expanded to include some recently characterized steps is[4–6]:



(1)

Kinetic studies using the isolated proteins indicate that ATP binds to the AM state (actomyosin with no bound nucleotide) and induces rapid detachment of the myosin head from actin[7]. We have used a laser photolysis method to study the corresponding reaction in muscle fibres capable of contracting. Muscle fibre tension and high frequency stiffness were monitored after rapid increases in ATP concentration to determine the kinetics of the mechanical events that follow binding of nucleotide to the cross-bridges.

Although solution experiments have demonstrated the reversibility of the actin–myosin association and dissociation reactions, and direct ATP hydrolysis without dissociation (shown above as dashed arrows)[8,9], for clarity of the experimental description, treatment of these phenomena is deferred to the discussion.

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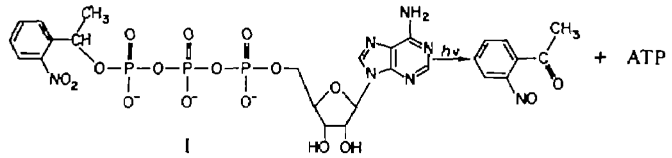
Photochemical initiation of cross-bridge detachment

The tension record of Fig. 1 illustrates the experimental procedure. A skinned single fibre from rabbit skeletal muscle was initially relaxed (solution 1). Transfer to a medium without ATP (solution 2) resulted in tension development as cross-bridges accumulated in the rigor (AM) state. The inert precursor of ATP, P^3-1-(2-nitro)phenylethyladenosine 5'-triphosphate, I, termed caged ATP[10], was added to the bathing medium (solution 3) and allowed to diffuse into the fibre. This caused little alteration of the rigor tension. At the arrowhead of Fig. 1 a pulse of UV radiation ($\lambda = 347$ nm) from a frequency-doubled ruby laser initiated the photolysis reaction[11]:



In the experiment shown in Fig. 1, 750 μM ATP was liberated in the muscle chamber. The rate of ATP release, set by photochemical dark reactions[11], was 100 s^{-1} in the solutions used in this study. Production of ATP in the presence of excess Mg^{2+} ions but no added Ca^{2+} resulted in a rapid decrease of tension to the relaxed level. Figure 1*b* shows the same tension decay on a faster time base. The arrowhead indicates the laser pulse. After a brief delay, partly due to the photochemical reaction, tension decreased with a half time ($t_{1/2}$) of ~5 ms. Thus ATP detaches cross-bridges rapidly in muscle fibres.

To make a kinetic comparison of cross-bridge detachment with the rate of actomyosin dissociation in solution, the amount of ATP liberated was varied by altering the intensity of the light pulse. Figure 2*a*–*c* shows transients of relaxation from rigor for several ATP concentrations. The upper traces in each panel are records of the high-frequency mechanical stiffness of the fibres, which is a qualitative indication of the number of cross-bridge attachments[12]. After the light pulses (arrows), the stiffness decayed, indicating cross-bridge detachment. Accompanying the stiffness decrease there was a delayed rise in force and then a decrease to the relaxed level (lower traces). As the ATP concentration was reduced, the peak in tension was delayed, the amplitude of the tension rise increased and the final relaxation was slower.

The time taken for the force to decay from the peak of the tension rise to half that value was converted to an apparent rate constant using the relation $k_{obs} = (\ln 2)/t_{1/2}$. This method of analysis ignores deviations of the final tension decay from a single exponential but is useful for comparison with the analogous experiment on actomyosin in solution. The rate of the final relaxation increased as the ATP concentration increased (see Fig. 2*d*). The slope of this relation, *K*, is a second-order rate constant for the overall detachment process of rigor cross-bridges induced by ATP. The average value for five fibres was $K = 1.9 \pm 0.6 \times 10^5\ M^{-1}\ s^{-1}$ (mean±s.d.) at 20 °C.

When acto-heavy meromyosin or actomyosin subfragment-1 is mixed in solution with ATP in a rapid reaction apparatus, and the dissociation of the myosin heads is monitored by decrease in light scattering, the apparent second-order rate constant for dissociation is $\sim 10^6\ M^{-1}\ s^{-1}$ (ref. 13, using acto-heavy meromyosin at ionic strength = 100 mM, $T = 20$ °C). Thus, our data indicate that in a fibre the rate of cross-bridge detachment from the rigor state is within an order of magnitude of the rate of the corresponding reaction with solubilized proteins.

The sensitivity of the detachment reaction to stress was tested by varying the length of the muscle fibre in rigor before photolysis. Figure 3 shows superimposed tracings of records obtained when small length steps were applied 1 second before the laser pulse. This altered the distribution of forces in the attached cross-bridges, which is apparent in the alteration of the shape of the initial 20 ms of the force transient. For Fig. 3*a*, the muscle fibre was stretched ~30 μm (0.86% of the initial length) before the laser pulse. Photolysis resulted in an initial rapid decrease in tension. The force imposed by the stretch was similar to the force reached during an isometric contraction of these glycerinated muscle fibres. No length change was applied for Fig. 3*b*. Figure 3*c*, *d* was recorded after the length was allowed to decrease. After the laser pulse, tension remained constant or increased before the final relaxation. Although the initial phase of these transients varies greatly in shape, the final phases superimpose.

Initial increase in tension

An initial tension rise was generally apparent if the tension was low (<50 mN mm^{-2}) at the time of the laser pulse. This low rigor tension level could be achieved either by decreasing the length of the muscle fibre just before the laser was triggered (for example, Fig. 3*d*) or by initially inducing rigor in a medium free of divalent metal ions and containing EDTA[14]. The initial rise in tension after liberation of ATP inside the myofibrils was

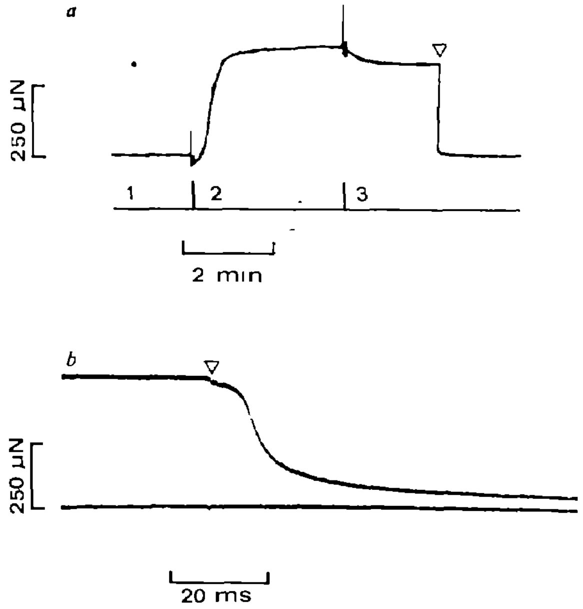


Fig. 1 Tension transient recorded from a single glycerol-extracted rabbit psoas muscle fibre. *a*, The slow time base tension record illustrates the experimental protocol. The fibre (cross-sectional area $7.95 \times 10^{-9}\ m^2$, sarcomere length 2.3 μm) was attached with aluminium clips between a force transducer (natural frequency, 7.5 kHz) and a piezoelectric stack which was used to alter muscle length. The fibre was immersed in a small trough, ~0.5 mm from a quartz window. Bars on the record indicate the timing of the solution exchanges. (1) Relaxing solution containing (in mM): MgATP, 0.1; Mg^{2+}, 1; K^+, 95; EGTA, 30; creatine phosphate (CP), 22; creatine kinase (CK), 1 mg ml^{-1}; TES (*N*-tris[hydroxymethyl] methyl-2-aminoethanesulphonic acid) buffer, 100; reduced glutathione, 10; ionic strength, 200; *p*H 7.1; 21 °C). (2) Rigor solution—same as (1) but without ATP, CP or CK. (3) Photolysis solution—same as (1), but without ATP and including 10 mM caged ATP. Photolysis of caged ATP was initiated at the arrowhead by a 150-mJ, 50-ns pulse of 347 nm light generated by frequency-doubling the output of a 694 nm ruby laser with a temperature-tuned rubidium dihydrogen arsenate crystal. After the rapid relaxation induced by the release of ATP, the photolysis solution was saved and later assayed for ATP using luciferin-luciferase[35,36]. *b*, Fast time base recording of the tension transient illustrated in *a*. The arrowhead indicates the time of the laser pulse. The lower trace is a baseline tension recorded after relaxation in the photolysis solution.

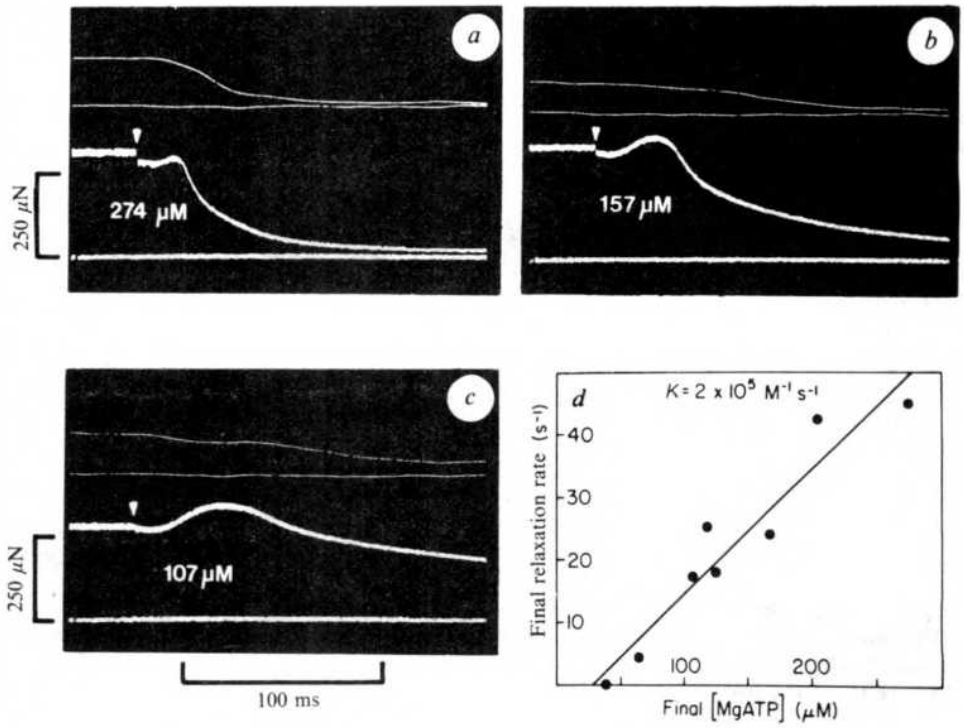


Fig. 2 Time course of relaxation at various concentrations of ATP. *a–c*, Paired stiffness (upper) and tension (lower) records. Lower traces of each pair show the baseline level in the relaxing solution. The degree of photolysis of the initial 2 mM caged ATP was controlled by varying the intensity of the light pulse[11]. Final ATP concentrations are shown for each panel. Stiffness was recorded by altering fibre length sinusoidally at 2.5 kHz and 1 μm amplitude with the piezoelectric stack. The sinusoidal component of the tension recorded in phase with the length change was separated from the d.c. component of tension by a synchronous demodulator. Nonlinear end compliance and sinusoidal phase shift due to stress relaxation may introduce small artefacts into the stiffness records. *d*, Observed rate of final relaxation of tension determined from the $t_{1/2}$ as described in the text. The data are from a single fibre. The line was fitted by linear regression, slope 2×10^5 M^{-1} s^{-1}. The non-zero intercept of the line is probably a reflection of the relatively high concentration of myosin heads within the fibre.

unexpected as mixing MgATP with actomyosin in solution causes rapid dissociation of the myosin head from actin filaments[3,7,13].

Figure 4*a* shows a control experiment with caged ADP that was performed to determine whether the shape of the photolysis-induced mechanical transient was caused by an artefact of the method. The photochemical species and accompanying reactions were identical with those in a caged ATP experiment except that 240 μM ADP was released. Little alteration in tension or stiffness occurred. However, when the liberated ADP was enzymatically converted to ATP by including creatine phosphate (CP) and creatine kinase (CK) in the medium, a tension rise and relaxation were observed (Fig. 4*b*). This indicates that these phenomena were caused solely by the appearance of ATP in the filament lattice.

A possible explanation of the initial increase in tension before relaxation that cross-bridges transiently reattach to the thin filaments shortly after detachment. If the reattached links produce more tension than the detaching cross-bridges, then tension increases. The stiffness (upper traces in Fig. 2*a–c* and Fig. 4*b*) declines during the initial tension rise, which indicates that the combination of detachment and reattachment results in a net detachment throughout the transient.

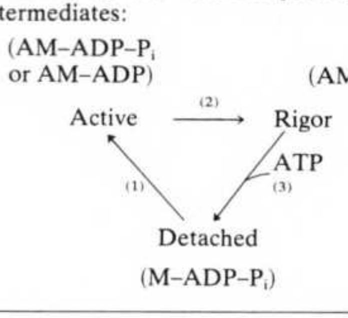
A rapid reattachment of detached cross-bridges might be allowed even in the absence of Ca^{2+} ions by a cooperative effect among thin filament attachment sites which has been demonstrated in other studies[15-17]. The dependence of the ATPase activity of rabbit myosin and regulated thin filaments (actin combined with troponin and tropomyosin) on ATP concentration and the relationship between the steady tension of skinned muscle fibres and ATP concentration are both bell-shaped with maxima at substrate concentrations of 2–30 μM[15-19]. These data can be explained on the basis of a cooperative interaction between thin filament attachment sites such that, if a proportion of cross-bridges are attached in rigor (nucleotide-free) bonds, then the remainder of sites are available for cross-bridge attachment irrespective of Ca^{2+} binding to troponin[15,16]. This cooperativity is consistent with the structure of the thin filaments, since tropomyosin regulates several actin monomers[20]. Interactions between the two heads of the myosin molecule might also account for cooperative attachment.

If cooperativity is to account for the tension rise, cross-bridges must be capable of detaching, reattaching and producing force within the 20–30-ms period between the illumination and the peak of the tension rise (Figs 2*a–c* and 3). The rate of reattachment and force generation can be tested by including Ca^{2+} ions in the photolysis medium, as liberation of ATP in the presence of Ca^{2+} will activate the muscle fibre to contract. If reattachment of cross-bridges occurs slowly compared with the detachment in this condition, then the muscle fibre should relax fully before the active high tension state is reached.

In Fig. 5 the tension record labelled $-Ca^{2+}$ shows a relaxation from rigor initiated by photolysis of caged ATP in the absence of Ca^{2+}. The length of the muscle fibre was allowed to decrease by 1% 1 s before the photolysis and a moderate concentration (190 μm) of ATP was generated in the fibre so that a significant tension rise would occur. In the next trial the fibre in rigor was bathed in a solution containing caged ATP and ~30 μM free Ca^{2+}. Addition of Ca^{2+} ions had little effect on the rigor tension or stiffness. When ATP was released in the presence of Ca^{2+}, the tension rose towards the level of an active contraction. The two force traces diverge within 20 ms after the laser pulse, indicating that the cross-bridges can generate additional force above the rigor level within 20 ms of ATP release. Assuming that rigor cross-bridges detach during this period, the results suggest that reattachment and force generation also occur rapidly.

Kinetic model

The possibility that a rapid reattachment of detached cross-bridges could account for the shape of the force transients was tested by using a restrictive model involving a minimum number of steps. Three cross-bridge states were considered in this calculation: detached, rigor and active. These are shown in the following scheme together with the probable corresponding chemical intermediates:



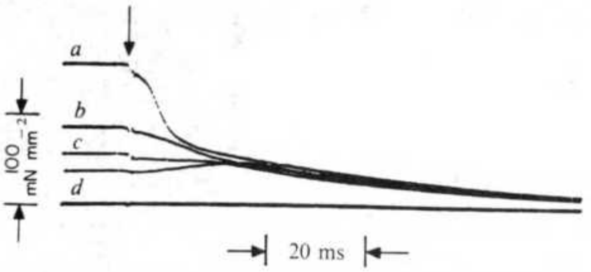


Fig. 3 Superimposed records of relaxation transients produced by photoliberation of ~480 μm ATP. The muscle length (3.5 mm; sarcomere length 2.32 μm) was changed slightly 1 s before the laser pulse. Length steps for each trace were: *a*, 30 μm stretch; *b*, no length step; *c*, 15 μm release; *d*, 30 μm release. Other conditions were as described in Fig. 1 legend. Units of tension per cross-sectional area are given for the force calibration to facilitate comparison with active contractions of glycerinated muscle fibres. The vertical positions of the records were adjusted to superimpose the four baselines (lower trace) showing the relaxed tension.

To introduce cooperativity into the model, the cross-bridge attachment sites were considered in pairs. For each member of a pair, reaction (1), the cross-bridge attachment step, was made to depend on the state of the other attachment. If one site of a pair was attached, then the attachment reaction at the other site was allowed (see Fig. 6 legend). For simplicity, the rate constants of these reactions were made independent of cross-bridge stress and strain. The resulting differential equations were integrated. The attached cross-bridge states were assigned values of force, given in Fig. 6 legend. The concentrations of the states, weighted by these relative forces, were summed to produce simulated curves of the tension time courses.

To simulate the experiments in which a stretch or release was applied to the muscle before the photolysis pulse (Fig. 3), the rigor cross-bridges present at the time of photolysis were given different values of force for each curve in Fig. 6. The shape of the initial portion of the curves depended strongly on the force per rigor cross-bridge. When the initial tension was low, detachment of rigor cross-bridges had a relatively small effect on the calculated tension curve and reattaching cross-bridges generated an increase in force as shown in the experimental records of Fig. 3*d* and 5 ($-Ca^{2+}$). For an intermediate initial tension (Fig. 6*c*), the effects of detaching and reattaching cross-bridges partially cancel, producing a delay similar to the experimental traces of Figs 1*b*, 2*a* and 3*c*. At high starting tensions, a briefer delay before relaxation (simulated trace, Fig. 6*a*; experimental trace, Fig. 3*a*) largely represents the photochemical dark reactions (at $100\ s^{-1}$) between absorption of the UV radiation and ATP release. Reattached cross-bridges were assigned a fixed weight so the later phases of the transients, dominated by reattachments, did not depend on initial rigor tension. This superimposition of the tension traces in the final phases of relaxation is a natural consequence of the reattachment hypothesis and is difficult to reconcile with other possibilities. Although other properties of the filament lattice may contribute to the observed shape of the transients[21], and the cross-bridge cycle in an active contraction may be kinetically very different, this model demonstrates that a cooperative attachment can explain the shape of the mechanical transients initiated by release of ATP within a rigor muscle fibre.

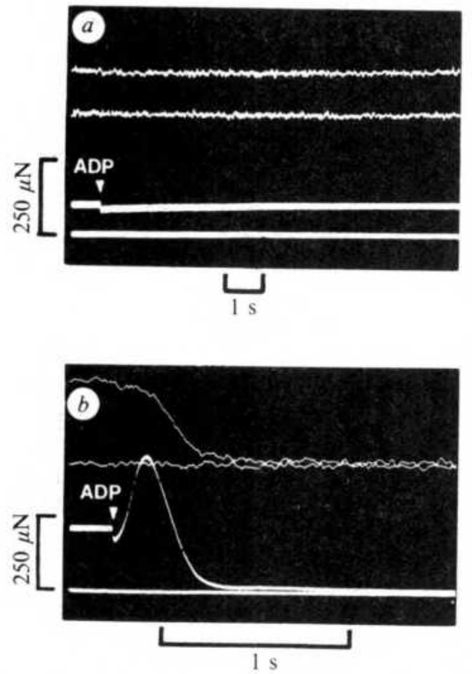


Fig. 4 Tension (lower) and stiffness (upper) records during photolysis of caged ADP. *a*, Caged ADP in the absence of CP and CK. Final ADP concentration was 240 μM. *b*, Photolysis of caged ADP in the presence of CP (22 mM) and CK ($1\ mg\ ml^{-1}$). Final ATP concentration, 290 μM. Conditions and baseline recordings (lower tension and stiffness traces) were as in Fig. 1 except that caged ATP was replaced by 2 mM caged ADP.

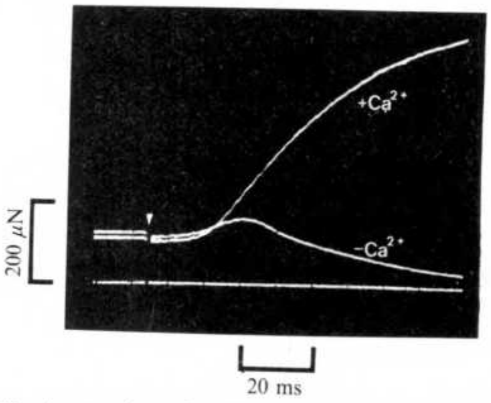


Fig. 5 Tension transients after liberation of 190 μM ATP in the presence ($+Ca^{2+}$) and absence ($-Ca^{2+}$) of free calcium. The muscle fibre (length 3.0 mm, sarcomere length 2.44 μm, cross-sectional area $4.2\times10^{-9}\ m^2$) was released by 30 μm 1 second before the laser pulse. Conditions were as described in Fig. 1 legend except that in the case of the $+Ca^{2+}$ trace, EGTA in the photolysis solution was replaced by 30 mM Ca-EGTA, to give a free Ca^{2+} concentration of ~30 μM.

Discussion

The observed rate of detachment of rigor cross-bridges in our experiments is whithin an order of magnitude of the rate of actomyosin dissociation in solution. This indicates that detachment from the AM state is not greatly slowed by the structural constraints of the filament lattice. The value of the detachment rate constant determined from the rate of the final relaxation over a range of released ATP concentrations ($1.9\times10^5\ M^{-1}\ s^{-1}$) is probably an underestimate of the actual rate as the final decay apparently includes some slow components related to reattaching and cycling cross-bridges. One approach to determining the pure rate of detachment of the rigor cross-bridges is to consider that a mechanical stretch or release of the muscle influences the force production of a particular cross-bridge only until it detaches. In that case, the computed tension difference between two of the traces in the pre-stretch and pre-release experiment (Fig. 3) decays at a rate reflecting the first detachment of the original rigor bonds. The traces converge by 10–30 ms, whereas the final decay of force and stiffness takes longer than 100 ms. This suggests that in muscle fibres the ATP-induced detachment occurs about as rapidly as with the solubilized proteins[3,7,13].

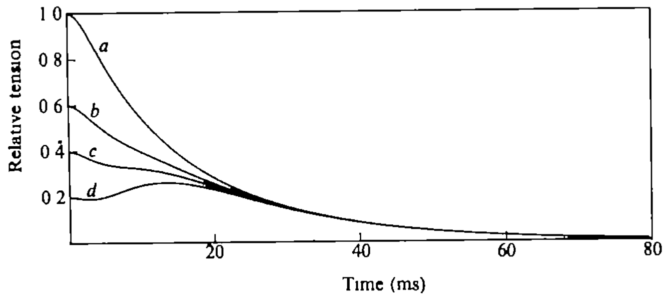


Fig. 6 Simulation of relaxation from rigor, including transient reattachment due to cooperativity between pairs of cross-bridges. Each cross-bridge of a pair was considered to be either in a rigor attachment, an active attachment or detached. Rigor states were further distinguished by whether they had existed continuously from the initiation of the simulation or whether they were formed from reattached active cross-bridges. In either case the second-order rate constant for detachment of a rigor cross-bridge by ATP was taken as $8\times10^5\ M^{-1}\ s^{-1}$. The reattachment rate was $50\ s^{-1}$ for those detached cross-bridges that had an attached partner. If both sites of a pair were detached, then no further attachment was allowed for that pair. The rate constant for transformation of active cross-bridges to rigor-type attachments was $100\ s^{-1}$. No stress- or strain-dependence was considered for any of these rates. Initial rigor cross-bridge concentration was 200 μM. To initiate the reaction, 500 μM ATP was introduced with a first-order rate constant of $100\ s^{-1}$. The resulting set of equations was integrated using the Gear method[37] to calculate how the concentration of each possible combination of pairs of cross-bridges varied with time. The forces generated by undetached rigor, active, and rigor bridges formed from active cross-bridges were given the following relative weights to generate curves of the simulated force transients after photolysis: *a*, 1, 3, 1; *b*, 0.6, 3, 1; *c*, 0.4, 3, 1; *d*, 0.2, 3, 1. Choice of these weights assumes that the state of reattached cross-bridges does not depend on the original rigor tension. Many features of the experimental records are apparent in this simulation.

Our experiments do not provide evidence concerning the extent of the direct hydrolysis pathway (dashed arrows in scheme 1) or the reversibility of the detachment step[8,9]. However, when the strain on a cross-bridge, induced by an applied stretch, is relieved on the first detachment, then the rapid convergence of the tension traces discussed above indicates the rate of the first detachment of the cross-bridges whether or not subsequent reattachment occurs before the hydrolysis step.

Detachment of cross-bridges from the rigor state with a $t_{1/2}$ of less than 10 ms (Fig. 1) is strikingly faster than the steady turnover rate of the actomyosin ATPase in isometric contractions (0.2–$1\ s^{-1}$; refs 19, 22–24). Thus, as a cross-bridge proceeds through the isometric contractile cycle, only a small proportion of time (<1%) is spent in the AM state. If the AM state which is present in rigor muscle fibres is the same as the nucleotide-free intermediate in the normal active cross-bridge cycle, then a cross-bridge which enters the AM state binds ATP and detaches within a few milliseconds.

During active shortening at forces less than the isometric value, the rate of energy liberation is three to five-fold higher than in an isometric contraction[25–27]. This control of energy liberation is not expected to occur at the step of AM dissociation as that reaction is not rate-limiting. This was confirmed by experiments in which stretches and releases were applied to the fibres in rigor before the laser pulse (Fig. 3); there was no decrease of detachment rate when the tension was increased into the range of physiological contractions.

Force transients in single muscle fibres which are initiated by a rapid change in length also indicate that the mechanics of the rigor state are different from the major force-producing states populated in an active contraction[28,29]. When an active muscle undergoes a small step decrease in length, the force drops and then recovers within the next few milliseconds to almost the original tension level[30]. This quick recovery phase may reflect the normal force generation process. The structural correlate of the steps between attachment and force generation might be a rotation or rocking motion of the myosin head towards the Z-line[2,30]. In rigor, force transients initiated by rapid length changes show much less quick tension recovery[28,29]. Thus, the motions or configurations available to the cross-bridge in its force producing state are not all available to the rigor cross-bridge. Several previous studies of rigor cross-bridge orientation, using C-ray scattering[31], fluorescence polarization[32] and electron paramagnetic resonance[33], have indicated that alterations of force caused by applied length changes do not rotate rigor cross-bridges.

Which intermediates would be predominant in a contraction? Our experiments show that cross-bridge detachment is too fast for sites to spend a large proportion of time in the AM (rigor) state. Unless Ca^{2+} markedly slows detachment from rigor, the result in the presence of Ca^{2+} (Fig. 5) indicates that the reactions of the detached cross-bridge and of reattachment are also too rapid to be limiting in an active contraction cycle. These data suggest that the rate limitation occurs between force production by the cross-bridge and rebinding of ATP. During a steady active isometric contraction, most of the cross-bridges would then be attached in the states that generate force. X-ray and mechanical data also support the hypothesis that most cross-bridges are attached during active isometric contractions[28,34]. The structural, mechanical, and kinetic data all suggest a model of the cross-bridge cycle in which the mechanical power transfer and control of energy liberation are separate from the detachment reaction and occur between corss-bridge attachment and the AM rigor intermediate.

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Functionally homologous cell cycle control genes in budding and fission yeast

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The cdc *2 (previously called* wee *2) cell cycle start gene of* Schizosaccharomyces pombe, *which is required for start and the control of mitosis, has been isolated from an* S. pombe *gene bank by complementation of a* cdc *2 mutation. A functionally homologous sequence which complements the* cdc *2 mutation has also been isolated from a* Saccharomyces cerevisiae *gene bank and this sequence has been shown to contain the* cdc *28 cell cycle start gene of* S. cerevisiae. *It is concluded that the* cdc *2 and* cdc *28 genes perform homologous cell cycle control functions in the two organisms.*

THE fission yeast *Schizosaccharomyces pombe* and the budding yeast *Saccharomyces cerevisiae* have both been used as model organisms for studying the eukaryotic cell cycle[1,2]. This work has made extensive use of temperature-sensitive *cdc* (cell division cycle) mutants which become specifically arrested during the cell cycle when incubated at their restrictive temperatures; over 40 *cdc* genes have been described in *S. cerevisiae* and over 25 in *S. pombe*. All of these are required for normal cell division but only a few are expected to be involved directly in cell cycle controls such as commitment to the mitotic cycle and regulation of the rate of cell division. In *S. cerevisiae*, four genes (*cdc* 28, 36, 37 and 39) have been shown to have roles in a major cell cycle control called 'start'[3-6]. Cells accumulate in G_1 at start in poor nutritional conditions and under the influence of the mating hormones. Mutants of the four *cdc* genes block cell cycle progress at start and are able to conjugate from their point of cell cycle arrest. Once start has been completed the cell becomes committed to the mitotic cycle and is unable to undergo the alternative developmental pathway of conjugation. Completion of start is also the major rate-limiting step of the cell cycle regulating the rate of cell division[3,4]. In *S. pombe* there are two start genes, *cdc* 2 and *cdc* 10 (ref. 7). Mutants of these two genes block in G_1 and are able to conjugate from that point of arrest. In poor nutritional conditions, *S. pombe* cells also accumulate before start. Thus the cell cycles of both organisms can be divided operationally into an uncommitted pre-replicative phase before start and a committed replicative phase consisting of S-phase, mitosis and cell division after start (Fig. 1).

Although start divides the cell cycles of the two yeasts into two similar phases this does not necessarily mean that the two start events have the same molecular basis in the two organisms, especially as they are not closely related. To establish whether the molecular basis of start is indeed similar we have made use of the fact that *cdc* start genes can be physically isolated by complementation and can be transferred from one yeast to the other. From these experiments we establish that *S. cerevisiae cdc* 28 can complement *cdc* 2 mutations of *S. pombe*, suggesting that the start events have a similar molecular basis in the two organisms.

Isolation of the *S. pombe cdc* 2 gene

A sequence containing a *cdc* start gene of *S. cerevisiae* has been selected from a bank of budding yeast DNA by its ability to complement the appropriate *cdc* mutation[8]. The development of techniques for high frequency transformation of *S. pombe* and the construction of a gene bank in a yeast–bacterial shuttle vector[9,10], makes the same approach feasible in *S. pombe*.

A *Hin*dIII partial *S. pombe* gene bank was made in vector pDB262, which contains the *S. cerevisiae leu* 2 gene and can complement the *leu* 1.32 mutation of *S. pombe*[9,10]. The gene bank was used to isolate the *cdc* 2 start gene by transforming a *cdc* 2.33 *leu* 1.32 strain to Leu$^+$ prototrophy and replica-plating the resultant 30,000 clones to 35 °C, at which temperature *cdc* 2.33 cells elongate and die (Fig. 2*b*; ref. 11). Clones that continued to divide at 35 °C were isolated and the plasmids which they contained were recovered in *Escherichia coli*[9,10]. One of these plasmids was found to complement the *cdc* 2.33 and *cdc* 2.M26 mutations when retransformed back into strains containing these mutations. The dividing cells in these transformant clones were elongated (Fig. 2*c*) compared with wild-type dividing cells (Fig. 2*a*), indicating incomplete suppression of the mutant phenotype by the insert on the plasmid. The plasmid was termed pcdc2.3(Sp) and contained a 7-kilobase (kb) insert. A 1.4-kb *Hin*dIII fragment from this insert was recloned into vector pDAM6 which contains the *S. cerevisiae leu* 2 and pBR322 but no sequence capable of supporting replication in *S. pombe*. This plasmid, pcdc2.32(Sp), was transformed into a *S. pombe leu* 1.32h$^-$ strain and some of the Leu$^+$ prototrophic transformants formed were found to be mitotically stable and were presumed to have arisen by plasmid integration into the chromosomal *cdc* 2 gene. This was confirmed for two of the integrants by preparing DNA from cells of the *leu* 1.32h$^-$ strain before integration and after integration of pcdc2.32(Sp). The DNA was digested with *Bam*HI, an enzyme which does not cut within the 1.4-kb *Hin*dIII insert but cuts once in the vector pDAM6 (Fig. 3). After Southern transfer the digested DNAs were probed with ^{32}P-labelled pcdc2.32(Sp) (Fig. 3). The 13-kb *Bam*HI fragment of DNA from cells before integration was altered to two fragments of 17 and 8 kb after integration for both integrants (see Fig. 3 for explanation). This result demonstrates that pcdc2.32 (Sp) integrated at the chromosomal site homologous to the 1.4-kb *Hin*dIII insert. We demonstrated that this site was the *cdc* 2 locus by showing that the Leu 1$^+$ marker phenotype of the plasmid was closely linked to *his* 3, a marker localized within 1 centimorgan of the *cdc* 2 locus[12]. The two pcdc2.32(Sp) integrants in strain *leu* 1.32 h$^-$ were crossed to a *his* 3$^-$ *leu* 1.32 h$^+$ strain. The resultant zygotes were sporulated and the phenotypes of 400 spores of both crosses examined. In both cases less than seven recombinant spores (*leu* 1$^+$ *his* 3$^-$ or *leu* 1$^-$ *his* 3$^+$) were observed, indicating that the *S. cerevisiae leu* 2 gene must have integrated within 1.7 centimorgans of *his* 3. Therefore, pcdc2.3(Sp) contains the *S. pombe cdc* 2 start gene.

Isolation of a *S. cerevisiae* sequence complementing *cdc* 2

Having shown that a sequence containing the *cdc* 2 gene could be isolated from *S. pombe*, we next tried to establish whether

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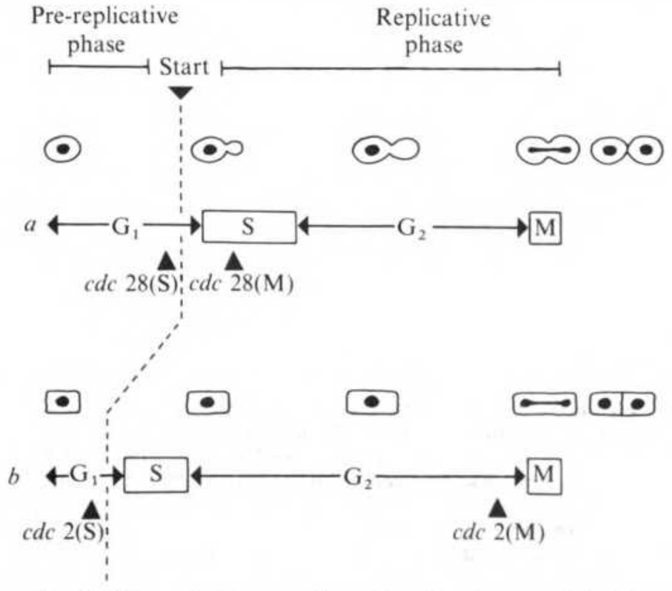

Fig. 1 Schematic representation of cell cycle control in *S. cerevisiae* (*a*) and *S. pombe* (*b*). Start divides the two cycles operationally into an uncommitted pre-replicative phase and a committed replicative phase. The pre-replicative phase occupies most of G$_1$. It is the major expandable part of the cycle, becoming much longer at slow growth rates. The figure represents rapidly growing cells in which the pre-replicative phase is longer in *S. cerevisiae* than in *S. pombe*. The transition or execution points of *cdc* 28 and *cdc* 2 are shown as filled triangles, the ones appropriate for start are labelled *cdc* 28(S) and *cdc* 2(S) and those appropriate for mitosis are *cdc* 28(M) and *cdc* 2(M).

there are any sequences in *S. cerevisiae* which have the same function as the *cdc* 2 gene. This can be done by introducing a *S. cerevisiae* gene bank into *S. pombe* and testing for complementation of the *cdc* 2.33 mutation. Previously, such complementation has only been observed for genes involved in intermediary metabolism such as *leu* 2 (refs 9, 10). A *S. cerevisiae* gene bank has been constructed in YEp13 (provided by K. Nasmyth), a vector which contains the *leu* 2 gene and can autonomously replicate in *S. pombe*. This gene bank was used to transform the *S. pombe* strain *cdc* 2.33 *leu* 1.32 in a manner identical to that described above for the isolation of pcdc2.3(Sp). A plasmid, pcdc2(Sc), was isolated which complemented the *cdc* 2.33 and *cdc* 2.M26 mutations. This plasmid contained *S. cerevisiae* sequences as its insert (data not given) and generated *cdc* 2$^+$ transformants having the same elongated appearance at the restrictive temperature as the *S. pombe* pcdc2.3(Sp) (Fig. 2*c*). Thus, *S. cerevisiae* contains DNA sequences which can functionally complement mutations in the *cdc* 2 gene.

S. cerevisiae cdc 28 can complement *cdc* 2

As *cdc* 2 is a start gene in *S. pombe*, pcdc2(Sc) might contain one of the four known *S. cerevisiae* start genes[5,6]. All of these have been isolated in the plasmids pcdc28, 36, 37 and 39 (ref. 8 and S. Reed, unpublished results) using vector YRp7 or its derivative YRp7′. The four plasmids were digested with *Hin*dIII and after Southern transfer were probed with ^{32}P-pcdc2(Sc). The hybridization observed in this experiment indicated that the inserts of pcdc2(Sc) and pcdc28 (containing the *cdc* 28 gene) contained common sequences. In particular, both plasmids had a 2.3-kb *Hin*dIII fragment in common. To test this was the same sequence, the 2.3-kb *Hin*dIII fragment was recovered from a digest of pcdc28, then nick-translated and hybridized to the similarly sized fragment from pcdc2(Sc) (Fig. 4). As the pcdc28 sequences are unique in the *S. cerevisiae* genome (S. Reed, unpublished results) this hybridization establishes that pcdc2(Sc) contains *cdc* 28 sequences. To confirm that pcdc2(Sc) contains a functional *cdc* 28 gene, the plasmid was transformed into a *cdc* 28.4 *leu* 2.3 *S. cerevisiae* strain (provided by I. Johnson). The resultant Leu$^+$ prototrophs grew at 36 °C, the restrictive temperature of *cdc* 28.4, demonstrating that pcdc.2(Sc) can complement a *cdc* 28 mutation and thus contains a functional *cdc* 28 gene. These experiments show that the *cdc* 28 gene contained within pcdc2(Sc) can complement mutations of *cdc* 2. To test whether the *cdc* 28 gene contained within pcdc28 will also complement mutations of *cdc* 2, the pcdc28 sequences first had to be transferred to a different vector because YRp7 cannot be selected for in *S. pombe*; pcdc28 was partially digested with *Sau*3A and ligated with *Bam*HI-digested pDB248. The crude ligation mix was used to transform the *cdc* 2.33 *leu* 1.32 *S. pombe* strain to Leu$^+$ prototrophy. About 30% of the transformants were able to form colonies at the restrictive temperature of 35 °C. The cells of these transformants were smaller (Fig. 2*c*) than wild-type cells (Fig. 2*a*) and were very similar to certain *wee* mutant alleles of *cdc* 2 (Fig. 2*d*). These *wee* mutant alleles have been interpreted as having an abnormally high *cdc* 2 gene product activity. Note that the behaviour of pcdc28 in *S. pombe cdc* 2.33 strains differs from that of pcdc2(Sc), which resulted in weaker complementation and the formation of elongated

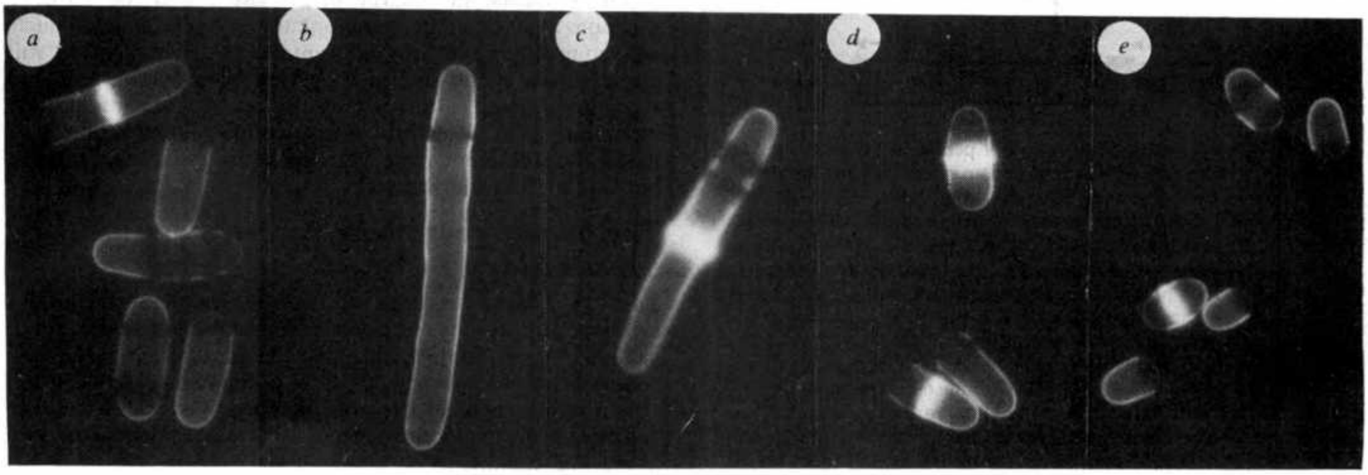

Fig. 2 Cells of *S. pombe* strains stained with Calcofluor and photographed by fluorescence microscopy. In these conditions the septa of dividing cells fluoresce intensely. *a*, Wild-type 972; *b*, *cdc* 2.33 *leu* 1.32; *c*, *cdc* 2.33 *leu* 1.32 containing pcdc2.3(Sp); *d*, *cdc* 2.1w (previously called *wee* 2.1); *e*, *cdc* 2.33 *leu* 1.32 containing pcdc28 in pDB248. Cells were grown in minimal medium at 25 °C overnight and were transferred to 35 °C in yeast extract medium for 7 h before staining as described in ref. 11. The cells in *e* include some dividing at a small size, similar to *cdc* 2.1w, and others which are highly elongated such as *cdc* 2.33 which have lost the plasmid and are unable to divide. pcdc2.3(Sp) is based on vector pDB262 which contains part of the yeast 2 μm circle, *leu* 2 and the bacterial plasmid pTR262. pDB248 is similar but with pTR262 replaced by pBR322[9,10].

cells (Fig. 2*c*). It is unknown why these two plasmids differ in their behaviour but as they were located in different vectors and were isolated from different gene banks the plasmid copy number and the flanking chromosomal and vector sequences could be different. Either of these two factors could influence the final activity of the *cdc* 28 gene product in *S. pombe*.

S. pombe cdc 2 cannot complement *cdc* 28

As *cdc* 28 can complement *cdc* 2 mutations of *S. pombe*, it was of interest to determine whether *cdc* 2 could complement *cdc* 28 mutations of *S. cerevisiae*. This was tested by transforming the *S. cerevisiae* strain *cdc* 28.4 *leu* 2.3 to Leu$^+$ prototrophy using pcdc2.3(Sp). The Leu$^+$ transformants did not grow at 36 °C, forming enlarged cells that were both budded and unbudded. This failure to complement indicates that the *S. pombe cdc* 2 gene cannot substitute for a defective *cdc* 28 gene in *S. cerevisiae*, which could be due to inadequate *cdc* 2 expression or inability of the *cdc* 2 gene product to provide the entire function of *cdc* 28. It has been shown that the 5′ regulatory sequences of the cytochrome *c* and alcohol dehydrogenase genes differ in *S. pombe* and *S. cerevisiae* (ref. 13 and P. Russell, personal communication). Thus the 5′ sequences of *cdc* 2 may be unable to initiate transcription at a sufficient level in *S. cerevisiae* to complement mutations of *cdc* 28.

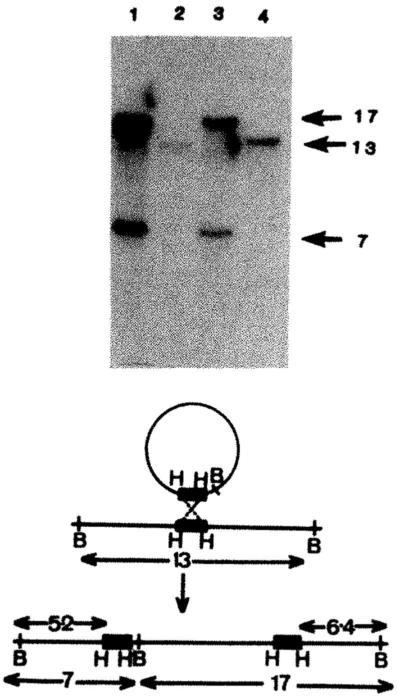

Fig. 3 A model of the integration of pcdc2.32(Sp) into the *cdc* 2 site on chromosome II (bottom) and an insert showing Southern blot transfers of *S. pombe* chromosomal DNA digested with chromosomal DNA (top) prepared from: tracks (1) and (3), pcdc2.32(Sp) integrants; tracks (2) and (4), the *leu* 1.32 strain before integration. B, *Bam*HI sites; H, *Hin*dIII sites. All procedures for DNA preparation, nick-translation with ^{32}P-CTP and Southern blot transfers are described in refs 9, 10. The fragments produced by integration can be explained as follows. The 1.4-kb *Hin*dIII fragment (shown as a thick line) derived from pcdc2.3(Sp) is contained within a 13-kb *Bam*HI chromosomal fragment. When pcdc2.32(Sp) of total length 11 kb integrates via homologous recombination at the *cdc* 2 site, a tandem duplication of the 1.4-kb *Hin*dIII fragment is formed which flanks the vector sequences of pDAM6. As pcdc2.32(Sp) contains only one *Bam*HI site situated in the vector next to the chromosomal insert, two new *Bam*HI fragments are generated with a combined length of 24 kb made up of the original *Bam*HI chromosomal fragment of 13 kb and pcdc2.32(Sp) (11 kb). The precise sizes of the two *Bam*HI fragments generated can be explained if it is assumed that the 1.4-kb *Hin*dIII fragment is located 5.2 and 6.4 kb from the chromosomal *Bam*HI sites.

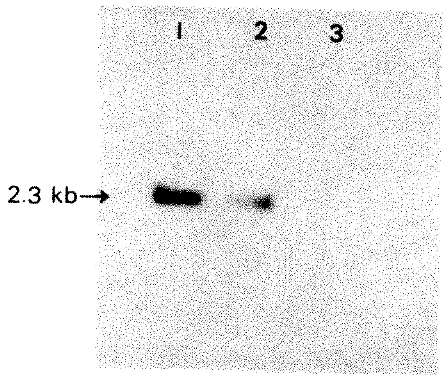

Fig. 4 Southern blot transfers of DNAs digested with *Hin*dIII and probed with the 2.3-kb *Hin*dIII fragment derived from pcdc28 (see text). Track (1), pcdc28; (2), pcdc2(Sc); (3), YEp 13. Note that a single 2.3-kb fragment hybridizes in pcdc28 and pcdc2(Sc) but there is no hybridization with YEp 13. All procedures for DNA preparation, nick-translation with ^{32}P-CTP and Southern blot transfers have been described elsewhere[9,10].

cdc 2 and *cdc* 28 genes perform similar functions in both G_1 and G_2

The requirement for the *S. pombe cdc* 2 gene in G_1 at start has already been described; *cdc* 2 is also required in G_2 before mitosis (Fig. 1). Whether cells become blocked in G_1 or G_2 depends on their position in the cell cycle at the time of temperature shift[7]. It has recently been shown that certain *cdc* 28 mutants of *S. cerevisiae* also arrest in G_2 just before mitosis as well as in G_1 at start[14]. Thus *cdc* 28 also has a dual role in the cell cycle at start and at mitosis, and is presumably providing both these functions when complementing *cdc* 2 mutations of *S. pombe*. This strongly suggests that the molecular function of the *cdc* 2 and *cdc* 28 genes is the same or very similar. To determine whether the *cdc* 2 and *cdc* 28 sequences are also closely related, pcdc2.3(Sp) was digested with *Hin*dIII and after Southern transfer was hybridized with ^{32}P-pcdc2(Sc). No hybridization of the inserts was detected in normal stringency conditions (0.75 M NaCl, 65 °C). This lack of sequence homology is not surprising as the two yeasts are not closely related and no hybridization was observed between *S. pombe leu* 1 and *S. cerevisiae leu* 2 genes, both of which encode the same enzyme, β-isopropylmalate dehydrogenase[9,10]. DNA sequencing will be required to establish the precise relationship between *cdc* 2 and *cdc* 28.

The molecular mechanism of the *cdc* 2 and *cdc* 28 gene functions at start and mitosis is unknown. As the gene products are required for two very different cell cycle events, they will possibly have some sort of triggering coordinating role. We envisage a molecular function such as that of protein modifications in the control of many other cellular processes[15], modulating different molecular activities at start and mitosis, but via the same mechanism.

The mitotic controls in *S. pombe* and *S. cerevisiae*

The *cdc* 2 gene function has been shown to be the major rate-limiting step in *S. pombe*, determining the timing of mitosis[16,17]. *wee* mutant alleles at the *cdc* 2 locus result in a cell cycle having a shorter G_2 than normal and a reduced cell size at mitosis and cell division. Before mitosis can occur, the cell must grow to a critical size and this requirement is reduced in the *wee* mutants[25,26].

In *S. cerevisiae* there is no mitotic control analogous to that found in *S. pombe*. Mitosis is thought to occur when a certain time has elapsed after 'start'[18,19]. The difference in control between the two yeasts may be related to the fact that mitosis is apparently initiated very early in the cell cycle in *S. cerevisiae*. A short intranuclear spindle forms by the end of G_1 (ref. 20). This structure elongates later in the cycle to form the full mitotic spindle during mitosis. The requirement of *cdc* 28 for mitosis is also completed early, about one-tenth of a cell cycle after start (Fig. 1; ref. 14). These observations suggest that the initiation of mitosis occurs early in the cell cycle in *S. cerevisiae*, a situation which is different from most eukaryotic cells such as *S. pombe*. When *cdc* 28 is introduced into *S. pombe* as pcdc28, mitosis is initiated prematurely so that cell division occurs at a small size (Fig. 2*e*). One explanation for this could be that the *cdc* 28 gene lacks some of the regulatory functions of *cdc* 2 which ensure that mitosis is delayed until the *S. pombe* cell has grown to normal size. *cdc* 28 may not have these functions because this control is not normally operative in *S. cerevisiae*.

Cell cycle control in other organisms

The *cdc* 2 and *cdc* 28 genes have major roles in the cell cycle control of *S. pombe* and *S. cerevisiae* and must perform identical or similar molecular functions. But the two organisms are not closely related as judged by both taxonomic criteria[21] and the comparison of 5S RNA[22] and cytochrome *c*[23] sequences, which show <70% homology. Despite this considerable evolutionary divergence, the molecular mechanisms of start and of the control initiating mitosis appear to have been highly conserved, thus we believe that these control mechanisms may also be applicable to other eukaryotes. In mammalian cells, G_1 restriction or transition points analogous to start in the yeasts have been proposed as the major point of cell cycle control[24]. Thus it is possible that the control in mammalian cells may also involve a functional equivalent of the *cdc* 2 and *cdc* 28 gene products.

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Arrangement of human immunoglobulin heavy chain constant region genes implies evolutionary duplication of a segment containing γ, ε and α genes

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Cosmid clones containing the human γ, ε *and* α *heavy chain constant region genes and an* ε *pseudogene have been isolated. All these genes have a switch sequence detectable by hybridization. We have studied overlapping cosmids covering two separate regions of the genome, and the gene order in each of these regions was found to be* γ–γ–ε–α. *This implies an evolutionary duplication in this multigene family involving* γ, ε *and* α *genes.*

MOST known mammalian genes are organized in multigene families whose members share sequence homology. Such families include the globin genes[1,2], interferon genes[3], growth hormone genes[4], genes of the major histocompatibility complex[5] and immunoglobulin genes. The immunoglobulin heavy (H) chain genes consist of four linked families of gene segments: the variable (V_H), diversity (D), joining (J_H) and constant (C_H) gene segments. The C_H gene segments determine the class of the H-chain polypeptide. Both man and mouse have five classes of H chain, known as μ, δ, γ, ε and α, which differ in their biological activities. In both species the γ chains are further divided into four subclasses, and in man there are two subclasses of α chain. When the active H-chain gene is formed by $V_H/D/J_H$ integration[6–8] the C_H gene closest to the J_H locus, the μ gene, is the first to be expressed while the other C_H genes can subsequently be expressed by H-chain switching mechanisms. One of these mechanisms, which operates for each C_H gene except δ, involves deletion of all C_H genes between the V gene and the newly expressed C_H gene[9–12]. This switch deletion has been proposed to involve recombination, perhaps by unequal sister chromatid exchange[13–15], between homologous, internally repetitive sequences (called switch or S regions) which were found upstream of each mouse C_H gene except δ[14,16–18]. It was similarly found in man that the μ gene S region has a repetitive sequence and is homologous with a region upstream of $\gamma2$ but not δ[19]. Comparison of S regions of mouse μ, γ and α genes with their human counterparts has shown strong evolutionary conservation [19–22].

The order of the C_H genes in the mouse has been determined as $5'$-μ-δ-$\gamma3$-$\gamma1$-$\gamma2b$-$\gamma2a$-ε-α-$3'$, and no pseudogenes have been reported[18]. The existence of two α-chain subclasses in man and the finding that human DNA contains a pseudo γ gene ($\psi\gamma$)[22,23] and three ε-like genes[24–26] implied that the arrangement of human C_H genes was different from that in the mouse. Some aspects of the arrangement of human C_H genes have already been published. The J_H–μ–δ region was mapped and found to be organized similarly to the mouse[19,27], the $\gamma2$ and $\gamma4$ genes were shown to be about 19 kilobases (kb)

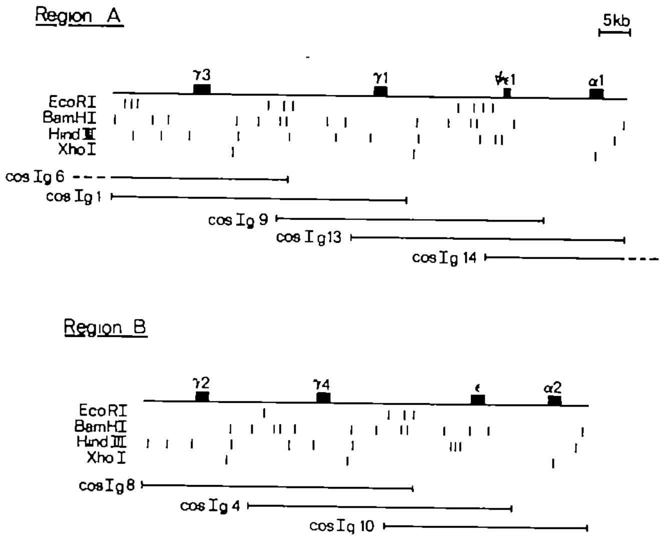

Fig. 1 Restriction maps of two regions of human DNA covered by overlapping cosmid clones. The cosmids were digested with the four enzymes shown, singly and in all pairwise combinations. Restriction fragments were separated on 0.8% agarose gels, and after reducing the fragment sizes by treatment with acid and then base[37], the DNA was blotted on up to four filters sequentially[38]. Each filter was hybridized to one of four probes: γ, ε or α gene probes as described in the text or the cosmid vector pOPF1 (F. Grosveld, personal communication). Overlaps between cosmids were confirmed by running all corresponding digests of overlapping clones in adjacent slots of agarose gels. The ends of cosIg6 and cosIg14 which extend beyond region A are shown as dotted lines because the restriction map in these regions has not been confirmed by comparison with other cosmid or phage λ clones.

apart[22,23,28], and it was reported that the ε and ψε1 genes are each linked to an α gene[24]. The ψε2 gene is apparently a processed pseudogene which has lost its intervening sequences, has a poly(A)-rich tail and has been conveyed to a new chromosomal location[24]. We wanted to study the organization of the human C_H genes further both to allow an evolutionary comparison with the mouse and also to allow a comparison of the H-chain genes of normal humans with those of individuals with immune deficiencies or leukaemias such as Burkitt's lymphoma. We present here a partial characterization of the C_H gene locus in man. Cosmid clones were isolated which apparently contained all the expressed γ, ε and α genes and ψε1. All of these genes have switch sequences which hybridize to S_μ. By mapping overlapping cosmid clones we identified two separate blocks of C_H genes, each with the order γ–γ–ε–α, implying an evolutionary duplication involving γ, ε and α genes. We propose an order of these two blocks of genes relative to each other and relative to the $J_H/\mu/\delta$ region.

Isolation and restriction mapping of cosmids containing γ, ε and α genes

Approximately 5×10^5 colonies of a human genomic cosmid library (F. Grosveld, personal communication) were screened with human γ and ε C_H gene probes. The γ probe was the 3.6-kb *Eco*RI–*Hin*dIII insert of p3.6RH4.2 (ref. 23) which contains the whole γ3 gene, while the ε probe was the 2.0-kb *Bam*HI–*Pst*I insert of ε1.2BP25 (ref. 25) which contains an entire ε gene. Several cosmid clones were isolated with these probes. Restriction mapping showed that the cosmids fall into two separate groups of overlapping clones, each group representing an unbroken region of chromosomal DNA, termed here region A and region B (Fig. 1). γ, ε and α genes were found in each region and located within the regions by hybridization to the γ or ε probes described above or to a mouse α cDNA probe consisting of the 0.6-kb *Eco*RI fragment from the 3′ end of the insert of p107αR5 (ref. 29). Both regions A and B were found to have a gene order γ–γ–ε–α. The total sizes of regions A and B are approximately 83 and 72 kb, respectively, and the distance between genes within each region ranges from 10 to 26 kb, which is similar to the spacing found between mouse γ, ε and α genes[18]. It is unlikely that the cosmids have undergone deletions because all parts of region A are covered by at least two cosmids, and although the terminal areas of region B are covered by only one cosmid, our map in those areas is corroborated by published maps of phage λ clones[22–26,28].

Characterization of genes present in cosmid clones

We obtained evidence by restriction mapping and hybridization as described above for four γ, two ε and two α class genes in the cosmids which we isolated. These genes were assigned to subclasses by sequencing or, in the case of some of the genes for which sequences were already available, by restriction mapping. However, we have not yet fully sequenced any of the genes present in these cosmids, so the subclass assignments remain provisional.

Restriction maps and partial sequences were known which distinguished the four γ subclass genes and the ψγ gene (U. Krawinkel and T.H.R., unpublished results and refs 22, 23, 28), so we could identify the γ genes in the cosmids on the basis of the restriction maps shown in Fig. 1. Apparently, the cosmids contain the γ1, γ2, γ3 and γ4 genes, but not ψγ. In the case of the γ1 and γ3 genes, this identification was confirmed by sequencing their distinctive hinge regions. Each hinge coding segment begins with a *Pst*I site[23], so *Pst*I fragments of cosIg1 and cosIg13 were subcloned into M13mp9 (ref. 30) and subclones containing hinge region fragments were selected by hybridization with the 1.8-kb *Pst*I fragment of p3.6RH4.2, which contains the fourth hinge of γ3. These clones were sequenced by the chain terminator method[31], giving sequences which agree with those previously published for the γ1 hinge coding segment and the four hinge coding segments of γ3 (data not shown). Identical γ1 hinge region sequences were obtained from cosIg1 and cosIg13, confirming the overlap between these two clones.

The ε gene in region B (Fig. 1) was identified as the active gene by restriction site comparison with the expressed ε gene of an IgE-producing myeloma, which is contained in the λ clone λε1.2 (ref. 25). Southern blot hybridization using the ε probe ε1.2BP25 showed that cosIg4, cosIg10 and λε1.2 have identical

Pseudoepsilon 1

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AAGCTTAGCTGGTTGGGCTGAGTAAGCTGGGCTGAGCTAAATGGGATTGAGCTGAGGAGG  60

GCTAGGCTGGGGGAGAGACCTGACGACGGACAGGGTTAAAAGCTGGAGTGAGCAGGCCTT 120

AAATTATTGAACTAAATTGGGCTGGGGTGATCTGAATTTAGCTGGGATGAGCTGGGCTGG 180

GCTGAACTGTGCCCACGTGAACTGGGCTAAACTAGGCTCGCCTGAGTGGACTCAGCTGGG 240

TTGGTCTCAACTGGGTTCAGCTGAGCTGGGCTCGGCTAGACTACACTGGGTTCAGCTGAC 300

                N  P  R  G  V  S  A  Y  L  S  R  P  S  P  F  D
ACTACACTGGGTTCAACCCGAGAGGGGTGAGCGCCTACCTAAGCCGGCCCAGCCCGTTCG 360

 L  F  I
ACCTGTTCAT
```

Fig. 2 Partial sequence of the ψε1 gene. *Hin*dIII–*Bam*HI fragments of cosIg13 were subcloned in M13mp8[30], and a subclone containing the 2.0-kb *Hin*dIII–*Bam*HI fragment with ε-like sequences was selected by hybridization with the insert of ε1.2BP25. This subclone was sequenced from the *Hin*dIII site by the chain terminator method[31], giving the sequence shown above. The region of the sequence which is identical to the active ε gene is underlined and surmounted by a deduced amino acid sequence in the single letter code.

a

```
 L  E  D  L  L  L  G  S  E  A  N  L  T  C  T  L  T  G  L  R  D  A  S  G [V] T  F  T  W  T  P  S  S  G  K  S  A  V  Q  G
CTCGAGGACCTGCTCTTAGGTTCAGAAGCGAACCTCACGTGCACACTGACCGGCCTGAGAGATGCCTCAGGTGTCACCTTCACCTGGACGCCCTCAAGTGGGAAGAGCGCTGTTCAAGGA  120

 P  P  E  R  D  L  C  G  C  Y  S  V  S  S  V  L  P  G  C  A  E  P  W  N  H  G [K] T  F  T  C  T  A  A [Y] P  E [S] K  T
CCACCTGAGCGTGACCTCTGTGGCTGCTACAGCGTGTCCAGTGTCCTGCCGGGCTGTGCCGAGCCATGGAACCATGGGAAGACCTTCACTTGCACTGCTGCCTACCCCGAGTCCAAGACC  240

 P  L  T  A [T  L  S] K  S
CCGCTAACCGCCACCCTCTCAAAATCCGGTGGGTCCAGAC  280
                            ↑
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b

```
 L  E  D  L  L  L  G  S  E  A  N  L  T  C  T  L  T  G  L  R  D  A  S  G [A] T  F  T  W  T  P  S  S  G  K  S  A  V  Q  G
CTCGAGGACCTGCTCTTAGGTTCAGAAGCGAACCTCACGTGCACACTGACCGGCCTGAGAGATGCCTCTGGTGCCACCTTCACCTGGACGCCCTCAAGTGGGAAGAGCGCTGTTCAAGGA  120

 P  P  E  R  D  L  C  G  C  Y  S  V  S  S  V  L  P  G  C  A  Q  P  W  N  H  G [E] T  F  T  C  T  A  A [H] P  E [L] K  T
CCACCTGAGCGTGACCTCTGTGGCTGCTACAGCGTGTCCAGTGTCCTGCCTGGCTGTGCCCAGCCATGGAACCATGGGGAGACCTTCACCTGCACTGCTGCCCACCCCGAGTTGAAGACC  240

 P  L  T  A [N  I  T] K  S
CCACTAACCGCCAACATCACAAAATCCGGTGGGTCCAGAC  280
                            ↑
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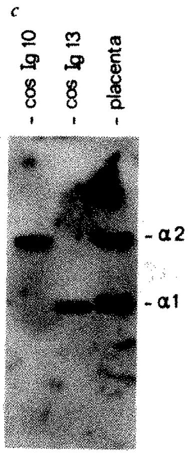

Fig. 3 Partial sequences of the putative $\alpha 1$ (*a*) and $\alpha 2$ (*b*) genes. *c*, Southern blot comparing α genes in cosIg10, cosIg13 and placental DNA. **Methods:** The 3.2-kb *Xho*I–*Hin*dIII fragments of cosIg10 and cosIg13 were subcloned in M13mp9[30] and sequenced from the *Xho*I site by the chain terminator method[31]. The deduced amino acid sequence is shown in the single letter code above the nucleotide sequence, and an arrow marks the splice site at the end of the C_H2 domain. Residues known to differ between the α_1 and α_2 proteins are boxed[32]. In *c*, all the DNAs were digested with *Pst*I, fractionated on a 1.4% agarose gel and blotted onto a nitrocellulose filter[39]. The DNA was then hybridized with 2×10^6 c.p.m. per ml of nick-translated α2XP8 at 65 °C with a salt concentration of $1\times$SSC and washed at 65 °C with $0.1\times$SSC[40]. The filter was then exposed to preflashed film at −70 °C with an intensifying screen[41].

sites for *Bam*HI, *Pst*I, *Sal*I, *Bgl*II, *Sma*I, *Pvu*II and *Hinc*II, at a total of nine sites distributed in and to either side of the ε gene. This result confirms the overlap between cosIg4 and cosIg10 and indicates that these cosmids contain the active ε gene which has been sequenced previously[24,25].

The ε gene detected by hybridization in region A (Fig. 1) was found by nucleotide sequencing to be an unusual type of pseudogene. Hybridization of the insert of ε1.2BP25, which contains all four coding segments of the active ε gene, was confined to a 2.0-kb *Hin*dIII–*Bam*HI fragment of cosIg13. This fragment was subcloned in M13mp8 (ref. 30) and sequenced, by the chain terminator method, 374 bases from the *Hin*dIII site (Fig. 2). For the first 313 bases this sequence shows homology to the S_μ region repeats but thereafter it is identical with the sequence of the active ε gene, except that it starts four bases into the $C_\varepsilon3$ coding segment. The $C_\varepsilon3$ segment therefore lacks an acceptor RNA splice site. We obtained other sequences homologous to $C_\varepsilon3$ and $C_\varepsilon4$ of the active ε gene by sequencing *Hpa*II fragments of cosIg13 (data not shown). Although the ε gene in cosIg13 contains $C_\varepsilon3$ and $C_\varepsilon4$ sequences, we found no evidence of $C_\varepsilon1$ or $C_\varepsilon2$ sequences by hybridization to the ε1.2BP25 probe. This gene thus seems to be a pseudogene which has lost the first two coding segments of an active ε gene by a deletion which fused an S-like sequence to a site just inside the $C_\varepsilon3$ coding segment. This deletion is apparently not a cloning artefact because the ε1.2BP25 probe hybridizes to a *Hin*dIII–*Bam*HI fragment of the same size in human genomic DNA and in cosIg13 (data not shown). Similar information was recently reported from studies on a phage λ clone, apparently describing the same ε pseudogene, which was called $\psi\varepsilon1$ (ref. 24). The structure of the $\psi\varepsilon1$ gene is strikingly analogous to the mouse myeloma IF2 mutant gene in which the internally repetitive switch sequences were first noted[14]. In both the $\psi\varepsilon1$ gene (which is a germ-line mutation) and the IF2 mutant (which is an *in vitro* mutant of a γ1-secreting myeloma cell line), sequences which are homologous to the S region have been joined to a site within a gene. In the former case this site is just within a coding region while in the latter it is within an intervening sequence, just before a coding region. Evidently, aberrant rearrangements involving S sequences are important in the formation of these mutations.

Preliminary identification of the α genes present in cosmid groups A and B was achieved by partial nucleotide sequencing and comparison of the derived amino acid sequences with the known $\alpha1$ and $\alpha2$ protein sequences. The mouse α probe described earlier (which contains only $C_\alpha2$ and $C_\alpha3$ sequences) hybridizes to a 3.2-kb *Xho*I–*Hin*dIII fragment of both cosIg13 (region A) and cosIg10 (region B). This fragment of each cosmid was subcloned in M13mp9 and more detailed restriction mapping of the subclones showed that in each case the *Xho*I site is within the α gene. The M13 subclones were therefore sequenced from the *Xho*I site towards the *Hin*dIII site, yielding the sequences shown in Fig. 3*a* and *b*. These sequences cover almost all of the $C_\alpha2$ domain, over a region where the $\alpha1$ and $\alpha2$ protein sequences are known to differ at seven characteristic amino acid residues[32]. Our deduced amino acid sequences for the genes in cosIg13 and cosIg10 correspond exactly to published protein sequences for $\alpha1$ and $\alpha2$, respectively[32], except for differences or ambiguities of amide assignment which are probably due to technical problems of protein sequencing. It therefore seems likely that the α gene in cosIg13 is $\alpha1$ and that in cosIg10 is $\alpha2$.

To confirm the hypothesis that cosIg13 and cosIg10 contain the active $\alpha1$ and $\alpha2$ genes we examined the number of α genes in human genomic DNA by Southern filter hybridization. Figure 3*c* shows a Southern blot comparing placental genomic DNA, cosIg13 and cosIg10, all digested with *Pst*I. The blot was hybridized to subclone α2XP8, which contains a 0.6-kb fragment of cosIg10 extending from the *Xho*I site near the start of $C_\alpha2$ to a *Pst*I site in $C_\alpha3$. The genomic DNA shows only two bands: a 2.0-kb band co-migrating with the band from cosIg10 and a 1.2-kb band co-migrating with the band from cosIg13. As no other hybridizing bands were detected, it seems likely that only two α genes exist in the genome and that the genes which we have cloned and partially sequenced are the active $\alpha1$ and $\alpha2$ genes. However, we cannot rule out the possibility that each band detected by Southern blotting in genomic DNA represents more than one gene. This caveat also applies to the γ and ε genes.

All the γ, ε and α genes have S regions homologous to S_μ

The H-chain class switch in antibody secreting cells is believed to be mediated by recombination between homologous S sequences near each C_H gene except δ. If this is correct we would expect to find that the human μ, γ, ε and α genes have homologous S sequences. We have studied this possibility by hybridization of an S probe, derived from the human μ gene, with human γ, ε and α genes. Figure 4 shows a Southern blot hybridization in which a restriction fragment containing S_μ was used to probe cosmids containing all the γ, ε and α genes described here and $\psi\varepsilon1$. As expected, all these genes, including

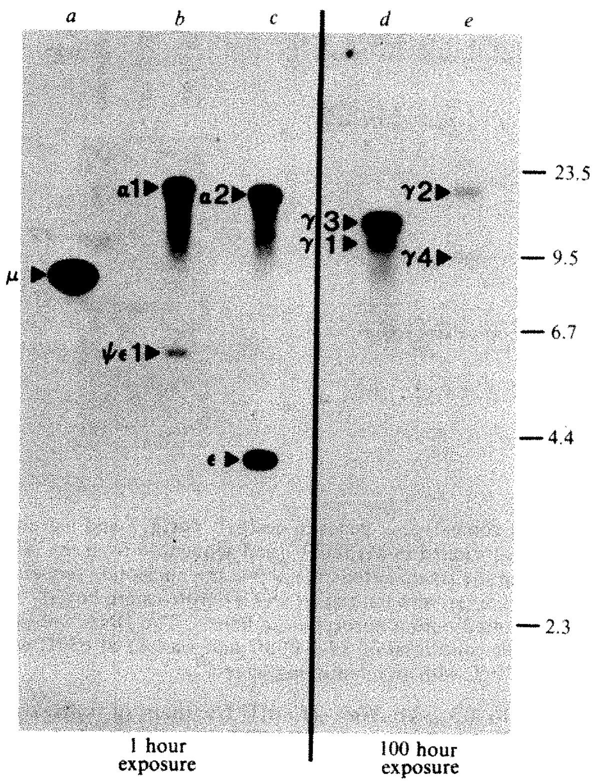

Fig. 4 Southern filter hybridization between an S_μ probe and S regions of other C_H genes. The probe was a 5.0-kb fragment of C76R51 (a subclone which contains the 8-kb *Eco*RI fragment from the 5′ end of λC76[19]) extending from an *Eco*RI site 45 base pairs inside the μ gene to a *Hin*dIII site in the 5′-flanking region of the μ gene. The restriction digests were as follows: *a*, cosIg1 × *Bam*HI; *b*, cosIg8 × *Bam*HI and *Eco*RI; *c*, cosIg10 × *Bam*HI; *d*, cosIg13 × *Bam*HI; *e*, C76R51 × *Eco*RI. Restriction fragments were separated on a 0.8% agarose gel and blotted onto a nitrocellulose filter[39]. The filter was then hybridized with 2×10^6 c.p.m. per ml of nick-translated probe in low stringency conditions: the hybridization and all washes were at 65 °C at a salt concentration of 6 × SSC[40]. The filter was then autoradiographed at −70 °C with an intensifying screen[41] for the times indicated.

$\psi\varepsilon_1$, have an S sequence which hybridizes to S_μ. This observation is consistent with a role for the S sequences in H-chain switching. However, even in the conditions of low hybridization stringency used here, S_μ hybridizes to S_γ very weakly—about two to four orders of magnitude more weakly than to S_ε or S_α. Among the γ genes, $S_\gamma 3$ hybridizes about 20–50 times more strongly with S_μ than do the other S_γ regions. Similar observations were made on mouse C_H genes[18], and the relative sequence divergence of S_γ raises questions about the H-chain switch mechanism. It has been suggested that sequences other than the S region repeats may be involved in switching[33], and it has also been proposed, on the basis of S-region homologies in the mouse, that γ genes are expressed initially by a switch from μ to the γ gene closest to μ, which can be followed by a switch from this γ gene to the other γ genes[34]. In support of this latter proposal, we find that in humans, as in mice, the γ gene which seems to be closest to μ (that is, the γ3 gene, see below) has the S_γ region which hybridizes most strongly to S_μ.

Order of the cosmid groups within the C_H locus

The cosmid clones described here fall into two groups which do not appear by their restriction maps to overlap either with each other or with the region around the μ and δ genes cloned in phage λ (refs 19, 27). However, we can predict the order of the two groups relative to each other and relative to the $J_H/\mu/\delta$ region by considering known deletions in an IgE-producing myeloma and two individuals with antibody deficiencies which are described in an accompanying paper[35]. It has previously been shown by Southern blotting that the IgE-producing myeloma 266B1 has undergone a deletion of ψε1 (refs 25, 26). During the H-chain class switch all C_H genes between J_H and the newly expressed C_H gene are deleted, so assuming that the deletion in myeloma 266B1 results from H-chain switching, ψε1 must lie between the J_H region and the active ε gene. Therefore, region A (containing ψε1) apparently lies between the $J_H/\mu/\delta$ region and region B (containing ε). Further support for this gene order comes from our accompanying paper, where we show that individuals lacking α1 antibodies and certain γ subclasses have a DNA deletion involving ψε1, the presumptive α1 gene and three γ genes (apparently γ1, γ2 and γ4)[35]. These data can be explained by a large inherited deletion which suggests the same order of the C_H genes as the deletion in the IgE-producing myeloma does. The overall gene order would therefore seem to be 5′–J_H–8 kb–μ–5 kb–δ– – – – γ_3–26 kb–γ1–19 kb–ψε1–13 kb–α1– – – – γ2–18 kb–γ4–23 kb–ε–10 kb–α2–3′. With the blocks of genes arranged in this way, all the genes have the same transcription orientation. The cosmid clones described here did not contain the two other known human C_H pseudogenes, ψε2 and ψγ, so we cannot comment on the location of those genes.

Evolution of the C_H gene locus

We have shown here that the C_H gene organization of man is strikingly different from that of the mouse in that human γ, ε and α genes are interspersed with each other, whereas in the mouse the four γ genes are clustered upstream of the single ε and α genes. This arrangement in man probably results from duplication of a large chromosomal region containing γ, ε and α genes. Two models for the evolution of the human C_H locus

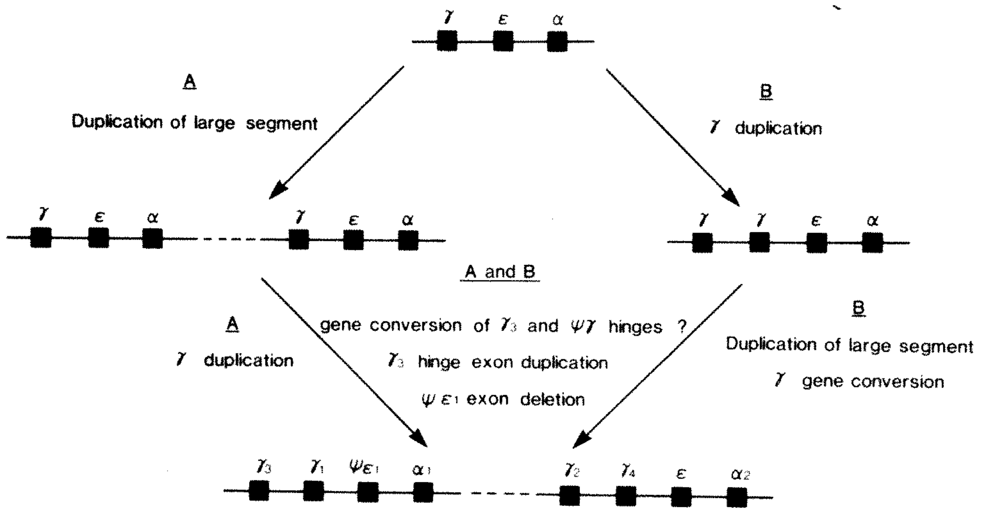
γ ε α
A
Duplication of large segment
B
γ duplication
γ ε α
γ ε α
γ γ ε α
A and B
gene conversion of γ3 and ψγ hinges ?
γ3 hinge exon duplication
ψε1 exon deletion
A
γ duplication
B
Duplication of large segment
γ gene conversion
γ3 γ1 ψε1 α1
γ2 γ4 ε α2

Fig. 5 Two models for the evolution of the human immunoglobulin C_H gene locus. The position of the ψγ gene is not known. ψε2 is apparently on a separate chromosome[24].

are illustrated in Fig. 5. The large ancestral segment which was duplicated may have had a gene order of either γ-γ-ε-α or γ-ε-α. In either case it is significant that when the protein sequences[36], gene sequences[22] and flanking restriction site patterns (Fig. 1) of the four human γ genes are compared, it is clear that $\gamma 3$ and $\gamma 1$ are particularly closely related to each other, as are $\gamma 2$ and $\gamma 4$. If a segment with the gene order γ-γ-ε-α duplicated (Fig. 5*B*), then the γ gene homologies suggest the operation of gene correction mechanisms such as gene conversion which have kept adjacent γ genes similar. Alternatively, the homologies can be explained by initial duplication of a segment with the gene order γ-ε-α, (Fig. 5*A*), followed by further duplications of the γ genes. γ_2 and γ_4 could have arisen by a simple gene duplication. The production of γ_3, γ_1 and $\psi\gamma$ is evidently more complex, because γ_3, unlike the other γ genes, has four hinge exons, the most 5′ of which is closely related to the single $\psi\gamma$ hinge exon while the other three are closely related to the single γ_1 hinge exon[22,23]. It has been proposed that $\gamma 3$ was created by an unequal cross-over between $\gamma 1$ and $\psi\gamma$ ancestors, thus implying a gene order $5'$-$\gamma 1$-$\gamma 3$-$\psi\gamma$-$3'$ (ref. 22). Our results do not seem compatible with this idea but suggest that exon duplication led to four γ_3 hinges originally all of the $\gamma 1$ type, followed by a gene conversion between the $\psi\gamma$ hinge and the first hinge of $\gamma 3$.

Several mechanisms have evidently contributed to the evolutionary diversification of the human immunoglobulin C_H gene cluster, including exon deletion in $\psi\varepsilon 1$, exon duplication in $\gamma 3$, possibly gene conversion between γ genes, duplication of individual γ genes and duplication of a large chromosomal segment containing three different types of gene—the γ, ε and α genes. An interesting possibility is the involvement of S segments or their precursors in certain evolutionary changes in this region such as the duplication of C_H genes. It may be possible to derive a more precise description of the events in the evolution of the C_H gene locus from a study of the DNA of other primates. In any case, the structure of the human C_H gene locus, which has emerged to be substantially different from that of the mouse, clearly reveals a high degree of evolutionary flexibility in the development of this multigene family.

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The roles of individual polyoma virus early proteins in oncogenic transformation

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The expression in normal rat cells of modified polyoma virus genomes, separately encoding large T, middle T or small T antigens, has allowed the investigation of the roles of these proteins in oncogenic transformation. Middle T is sufficient to transform cells of established lines but the transformants are serum dependent. Large T lacks intrinsic oncogenic potential but can relieve the serum dependence of normal and transformed cells. Middle T alone cannot transform primary rat embryo fibroblasts.

GENETIC analysis of the respective roles in oncogenic transformation of the three known polyoma virus early proteins, large T, middle T and small T[1–4], is complicated by the overlapping organization of the coding sequences in the viral DNA[5–7] (Fig. 1). The superimposed information content of the genomic DNA, contained in alternate translational reading frames, is resolved *in vivo* by differential processing of a common primary early region transcript: single introns of different lengths and positions are excised to produce three distinct mRNAs which individually encode the proteins. Application of the contemporary gene transfer techniques to study the biological functions of the viral early proteins would be facilitated by prior resolution of the coding information at the DNA level. This was achieved initially for the middle T protein[8] and more recently for the large T and small T proteins[9,10] by precise deletion of intron sequences from otherwise full-length viral DNA cloned in a plasmid vector. Introns were removed by replacing genomic restriction fragments spanning them with the corresponding shorter fragments derived from cDNA clones[11]. The three

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Table 1 Structure and coding capabilities of the recombinant plasmids

Plasmid	Polyoma DNA insert	Deletion (nucleotide)*	Coding capability Large T	 Middle T	 Small T
pPY1	*Bam*HI genomic	None	+	+	+
pPyLT1	*Bam*HI genomic	Large T intron (410–794)	+	−	−
pPyMT1	*Bam*HI genomic	Middle T intron (747–808)	−	+	−
pPyST1	*Bam*HI genomic	Small T intron (747–794)	−	−	+
pNG1	*Bam*HI genomic	NG18† (512–698)	+	−§	−§
pMC1	*Bam*HI-*Eco*RI	NG18† (512–698)	Truncated amino terminal‡	−§	−§
pLT214	*Bam*HI-*Eco*RI	Large T intron (410–794)	Truncated amino terminal‡	−	−

* Nucleotide numbers in the polyoma DNA sequence according to ref. 37. † See refs 23–26. ‡ See text.

§ The common N-terminal polypeptide expected from translation in the middle T/small T frame up to the out-of-phase deletion (~10,000 daltons) has not been unambiguously identified in cells infected with the NG18 mutant, although mRNAs with the appropriate splices are synthesized (our unpublished results and G. Carmichael and T. Benjamin, personal communication).

recombinants generated (Fig. 1, Table 1) were designated pPyLT1, pPyMT1 and pPyST1 to indicate the intron deleted. Separate transfer of the modified genomes into cultured cells results in the production of the expected single early protein (refs 8, 9 and Z. Zhu, G. M. Veldman, A. C., A. C. and R. K., manuscript in preparation) through the efficient expression of unspliced mRNAs.

Many studies on polyoma virus-induced transformation[12–20] have pointed to a critical role for the middle T protein, without excluding an involvement of all or parts of the other two early gene products. Experiments done with temperature-sensitive mutants of the large T protein have indeed suggested a role for the amino-terminal region of this protein in the maintenance of the transformed state in cell lines derived from FR3T3 rat cells[21]. The first set of results obtained with the modified viral genomes encoding only one of the early proteins showed that DNA of the middle T plasmid pPyMT1 transformed rat cells of the F2408 established line only slightly less efficiently than did cloned wild-type DNA. Transformed lines were obtained which synthesized the middle T, but neither the large T nor the small T protein. They grew in agar and to high density on plastic when tested in medium supplemented with 5% fetal calf serum. The 'middle-T-only' lines assayed for oncogenicity all induced tumours in young Fischer rats in a manner indistinguishable from that of control lines producing all three early proteins. Although it was therefore clear for the first time that middle T expression alone is sufficient to initiate and to maintain an oncogenic state, significant alterations in cell physiology caused by the expression of large T and/or small T were still not exlcuded. It was suggested[8] that if middle-T-only transformants could be shown to differ from other polyoma virus-transformed cells in some selectable property, it might be possible to assay for functions of the other viral proteins by transfer into the cells of appropriately modified genomes. We report here that such a property does exist. Cells transformed by plasmid pPyMT1 have a conditional defect in the expression of the tranformed state: they revert to an apparently normal phenotype when grown in low serum medium. We show that the ability to maintain transformation at low serum concentration, which is characteristic of cells transformed by wild-type viral DNA, is specifically complemented by modified viral genomes that encode either all of the large T protein or only its N-terminal 40%. The same DNAs, while lacking transformation potential, could be used to derive FR3T3 lines with substantially reduced serum dependence. These results motivated us to ask whether the requirements for transformation of primary embryo fibroblasts differ from those of established cells lines. Successful transformation of these cells was achieved with cloned wild-type DNA, but not with any of the modified genomes separately encoding the early proteins.

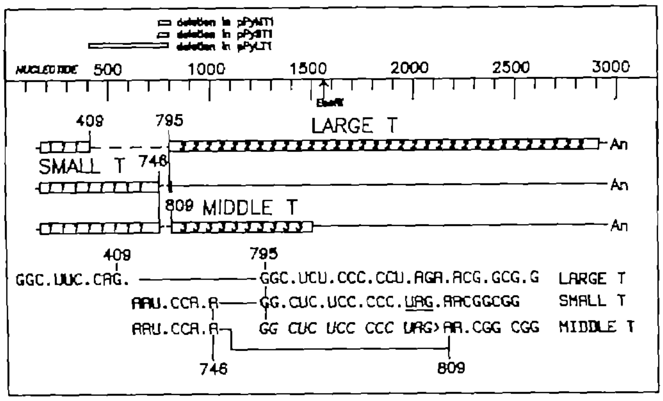

Fig. 1 Expression of the polyoma virus early region. A linearized map of the early region DNA is shown, marked with conventional nucleotide numbers[37] and the reference cutting site for endonuclease *Eco*RI. The three different spliced mRNAs are aligned with the map Coding regions are boxed, non-translated regions are represented by continuous lines and introns by dashed lines. Numbers within the coding regions indicate the translational reading frame used; note that the 3′ exon of the small T protein mRNA is translated in frame 1. RNA sequences across the splice junctions are shown in the lower part of the figure to illustrate how the joining of the two 5′-splice sites with two 3′-splice sites in three of the four possible combinations accomplishes the changes in codon reading frame required to use the two open frames available between nucleotides 809 and 1,497. The positions of the specific intron deletions in the viral genomes of plasmids pPyMT1, pPyLT1 and pPyST1 (see Table 1) are illustrated at the top of the figure.

Transformation of FR3T3 cells by DNA encoding only the middle T protein

The transforming activities of recombinant plasmids (Table 1) containing genes uniquely encoding large T (pPyLT1), middle T (pPyMT1) or small T (pPyST1) were tested with a Fischer rat cell line of the 3T3 type[22], FR3T3[23]. A recombinant containing unmodified viral DNA (pPY1) served as the control. DNA was transferred into cells by fusion with bacterial protoplasts[24]. Frequencies of transformation were measured by focus formation in medium containing 10% calf serum (Table 2). As expected from previous results with *hrt* mutants[25–27] (which express only the large T protein), plasmid pPyLT1 did not transform. Plasmid pPyMT1 transformed FR3T3 cells with a somewhat lower efficiency than the control plasmid pPY1, a result similar to that previously reported for F2408 cells[8]. Plasmid pPyST1, which encodes only the small T protein, did not transform. Additional plasmids were also negative: pNG1[21], including the entire genome of the partial large T intron deletion mutant NG18 that expresses only the large T protein[25–27]; pLT214, including a fragment of the viral DNA insert from pPyLT1 spanning the 5′ half of the early region and encoding an N-terminal truncated form of the large T protein (334 virus-coded amino-acid residues plus 16 C-terminal residues from vector sequences); pMC1[21], including the corresponding fragment from pNG1 and also encoding a truncated large T protein. Data shown in Table 2 for primary rat embryo fibroblast cultures will be discussed below.

Serum-dependent growth properties of transformants expressing only middle T protein

As illustrated in Fig. 2, FR3T3 cells grow to the monolayer stage in medium supplemented with 10% calf serum (*a*), but are unable to divide in only 0.5% serum (*b*) and die in growth medium without serum (*c*). Transformation with wild-type polyoma virus DNA, represented in Fig. 2 by the PYT21 line, derived by transfer of plasmid pPY1, enables the cells to grow past the monolayer stage to high density in either 10% (*a*) or 0.5% (*b*) serum, and also to grow relatively well without exogenous serum factors (*c*). By contrast, all lines derived from FR3T3 by transfer of pPyMT1 ('MTT' lines) grew to high density in 10% serum (*a*), but stopped at confluence in 0.5% serum (*b*). Even with frequent medium changes, these cultures could be kept at the monolayer stage for at least 2 weeks: neither cell death nor cell division could be detected by microscopic examination. The cells became larger and expanded on the plastic surface, a morphology reminiscent of that of the normal cells and different from that of wild-type polyoma virus transformants and of the same MTT cells in high serum medium. On addition of 10% calf serum, these cultures resumed their growth up to the high cell densities characteristic of transformed lines (data not shown). When serum was completely omitted, wild-type transformants could still grow, but MTT cells either grew very poorly or died over a period of 5–7 days (Fig. 2*c*). Below a critical serum concentration, cells which express only middle T thus have the growth properties on plastic of non-transformed cells. This also applies to their ability to divide in the absence of anchorage : all MTT lines tested so far exhibited a high cloning efficiency in agarose medium containing 10% serum (see also ref. 8), but, unlike cell lines transformed with wild-type viral DNA, none of them could form even microcolonies when the serum concentration was reduced to 0.5% (Table 3). This differential serum requirement of cells expressing only middle T has been confirmed without exception using other FR3T3-derived MTT lines, as well as middle-T-only lines derived from F2408 (see Table 3, ref. 28 and P. Kaplan and B. Ozanne, personal communication).

Table 2 Efficiency of transformation of FR3T3 rat cells and of rat embryo fibroblasts by plasmids encoding individual polyoma virus early proteins

	Foci per plate		
		Rat embryo fibroblasts	
Plasmid	FR3T3 cells	Expt 1	Expt 2
pBR322	0	0	0
pPY1	128	56	256
pPyLT1	0	0	NT*
pNG1	0	0	NT
pPyMT1	90	0	0
pPyST1	0	0	NT
pMC1	0	NT	NT
pLT214	0	0	NT

Confluent primary cultures, prepared as described in ref. 38 from 14-day-old Fischer rat embryos and parallel cultures of an early passage of the FR3T3 line[21] were seeded at a density of 2×10^5 cells per 35-mm Petri plate (Lux) in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum (Gibco). After 24 h, the cells were fused with bacterial protoplasts (5×10^3 to 10^4 protoplasts per cell) of *E. coli* 1106 bacteria carrying the indicated plasmids, according to our previously published procedure[22]. Three days later, cells were trypsinized and plated at a density of 10^5 cells per 35-mm Petri plate. Cultures were stained with Giemsa and foci were counted after 10 days for FR3T3 cultures and after 1 month for rat embryo fibroblast cultures (no further increase in the number of foci was observed after 8 weeks incubation of parallel plates for both types of cultures). Numbers are total foci per 8×10^5 FR3T3 cells, 4×10^5 rat embryo cells in expt 1 and 2×10^6 in expt 2. NT, not tested.

Table 3 Growth in agarose medium of normal, wild-type polyoma-transformed and pPyMT1-transformed rat lines

	Colony-forming ability in agarose medium supplemented with:	
Cell line	10% Calf serum	0.5% Calf serum
FR3T3	<0.001	<0.001
PYT21	100	14
4.2	100	20
MTT1	100	<0.001
MTT4	100	<0.001
2.9	100	<0.001

10^5 cells of the indicated line were seeded in agarose medium supplemented with the indicated amount of serum. Microcolonies per microscope field were counted after either 8 days (10% serum) or 15 days (0.5% serum) and values were normalized as per cent of the cell input. The origin of cell lines MTT1, MTT4 and PYT21 is indicated in Fig. 2 legend. The same results were obtained for three other MIT lines independently isolated from FR3T3 (MTT2, MTT3, MTT5). Transformed lines 4.2 and 2.9 were derived[8] from F2408 Fischer rat established fibroblasts after transfection with plasmids containing either wild-type (4.2) or pPyMT1 polyoma (2.9) DNA.

Can serum-dependent MTT transformants be complemented by other viral proteins?

To assess whether the serum-dependent transformed phenotype of MTT transformants could be complemented by one of the other early viral proteins, MTT4 cells were fused with bacterial protoplasts carrying various plasmids, seeded in medium containing 0.5% serum and submitted to selection for stable transformants (Table 4). Colonies in agarose medium were observed, not only after transfer of a complete transforming region, but also with plasmids pNG1, pLT1, pMC1 and pLT214, that encode either a full-size or a truncated large T protein and cannot by themselves transform FR3T3 cells (see Tables 2, 5). Similar complementation results were obtained when focus formation at low serum concentration was used as the transformation assay (data not shown).

Several agar colonies and foci resulting from complementation were picked and grown into cell lines at low serum concentration. They all now had the growth properties characteristic of wild-type transformants. Representative cell lines, complemented with the pPyLT1 plasmid, were tested for the expression of the large T protein: unlike the parental MTT4 cells, they exhibited the nuclear staining by immunofluorescence with anti-T antigen serum characteristic of the large T protein (data not shown) and immunoprecipitation of T antigens demonstrated the expression of a full-size large T protein (Fig. 3*a*). A novel immunoprecipitable polypeptide could be detected in MTT cells complemented by DNA encoding only the N-terminal 40% of the large T polypeptide (plasmid pMC1, see Table 1) (Fig. 3*b*). Like the full-size large T protein, it could be labelled *in vivo* with ^{32}P-orthophosphate. Its apparent molecular weight (40,000) closely corresponds to the size predicted from the DNA sequence (36,700) and is identical to that of an immunoprecipitable polypeptide previously identified in a cell line (FR3T3-BR70, see below) isolated after transfer of lasmid pMC1 in FR3T3 cells[21]. Although further characterization of this protein is required, these results support the genetic evidence that the activity of a truncated large T protein can complement the serum dependence of MTT transformants.

No efficient complementation was observed in the progeny of MTT cells fused with pPyST1 protoplasts (Table 4). However, prolonged incubation of these plates produced a significant number of very small colonies in agarose medium, or foci on plastic plates. A representative number of these colonies were picked, but we have been unable to establish from these cells lines able to grow in low serum medium.

Selection of transformants at low serum concentration

The results described above suggested that pPyMT1 DNA alone was unable to transform FR3T3 cells if transformation were monitored by growth at low serum concentration[29]. We would further expect transformation in such conditions to be complementable by plasmids able to rescue the serum factor dependence of MTT transformants. In the experiment reported in Table 5, FR3T3 cells were fused to pairs of protoplasts carrying either pBR322 or pPyMT1 plus other viral recombinants or pBR322. Transformed colonies were quantified in agarose medium containing either 10 or 0.5% serum, and focus formation was measured in liquid medium containing 0.5% serum. The results confirmed both expectations. Transfer of pBR322 plus individual polyoma recombinants yielded transformation in 10% serum with genomic (pPY1) or middle T (pPyMT1) plasmids, but in 0.5% serum, only with the pPY1 genomic plasmid. Complementation of pPyMT1 transformation in 0.5% serum was clearly observed with the recombinant genomes that encode at least the amino-terminal 40% of the large T polypeptide (pPyLT1, pNG1, pMC1). Transfer of pPyMT1 plus the plasmid that encodes only the small T protein (pPyST1) did not yield visible foci in low serum medium, except after prolonged incubation (4 weeks) of the plates, where several very small foci were found. This effect of pPyST1, similar to that observed in the complementation of MTT cells (see above), also requires further study.

Selection for growth at low serum concentration in the absence of the middle T protein

Our complementation data showed that the characteristic very low requirements for serum growth factors of polyoma virus-transformed cells is under the genetic control of the large T gene. To test whether this phenotype can be acquired without expression of the middle T protein, selection for growth at low serum concentration was applied after transfer of the plasmids that code for the amino-terminal end of large T into cells of the highly serum-dependent FR3T3 line (see Fig. 2). FR3T3 cells, after transfer of vector DNA alone (Table 6), had a very low plating efficiency ($\sim 2\times10^{-3}$) in medium supplemented with 0.5% serum. This was increased by 5–10-fold (Table 6) when the cells had been fused with protoplasts carrying either pPyLT1 (or pNG1) or pLT214 (or pMC1). These clones could be further grown in low serum conditions into cell lines. In the presence of 10% serum, these lines had growth characteristics similar to those of the parental FR3T3 cells: they retained a low saturation density (Table 6) and did not grow in suspension. They could, however, be differentiated from the parental cells by their ability to grow to a low saturation density in the presence of 0.5% serum; curiously, the growth of such lines in low serum medium was similar to that of MTT lines in the same conditions (Table 6). Immunofluorescence measurements on representative lines derived after transfer of pPyLT1 showed positive nuclei characteristic of the expression of the large T protein. This is consistent with the conclusion that, at least after transfer of the entire large T gene, the modification in serum requirement results from the expression of a large T related polypeptide.

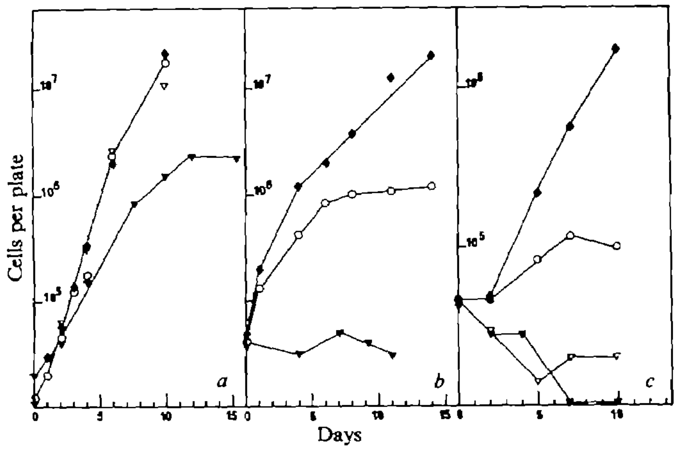

Fig. 2 Growth of normal FR3T3 cells and representative MTT and wild-type polyoma transformants at various serum concentrations. Cells were seeded at a density of 5×10^4 cells per 60-mm Petri plate in DMEM supplemented with either 10% (*a*) or 0.5% (*b*) serum or without addition of serum (*c*). The medium was changed every other day below 10^6 cells per plate and every day above this value. At the indicated times, plates were trypsinized and counted. MTT1 and MTT4 lines were derived from FR3T3 cells by focus formation in medium containing 10% serum after transfer of plasmid pPyMT1. PYT21 was established in a parallel experiment after transfer of plasmid pPY1. ▼: FR3T3; ◆: PYT21; ▽: MTT1; ○: MTT4.

Table 4 Complementation of MTT cells for growth in agarose medium at low serum concentration

Fusion of MTT4 cells with protoplasts carrying	Colonies per 10^5 cells 17 days	7 weeks
pBR322	0	0
pPY1	30	47
pPyMT1	0	0
pNG1	20	38
pPyLT1	8	32
pMC1	12	20
pPyST1	0	15*
Control without fusion	0	0

MTT4 cells were seeded (2×10^5 cells per 35-mm plate) in DMEM containing 0.5% serum. They were fused the next day with 10^4 protoplasts per cell of *E. coli* 1106 bacteria carrying the indicated plasmids. After 24 h, cells were trypsinized and seeded (10^5 cells per 60-mm plate) in agarose-gellified DMEM containing 0.5% newborn calf serum. Colonies were first counted after 17 days and again after 7 weeks. Numbers are averages of six plates.

* Minute colonies of slow-growing cells.

In an independent experiment[21], plasmid pMC1 (see Table 1) was transferred into FR3T3 cells and progeny colonies, grown in 10% serum, were randomly screened for the expression of a viral nuclear T antigen. Out of the 77 clones tested, 6 were weakly positive and one of them, FR3T3-BR70, was kept for further analysis. These cells were shown[21] to synthesize a protein immunoprecipitable with anti-polyoma virus T antigen, with the expected molecular weight of 40,000, identical to that of the antigenic protein now observed in MTT cells complemented by pMC1 plasmid (see above Fig. 3). The cells had the same morphology, growth inhibition in suspension and low saturation density (Table 6) as the original FR3T3 cells when studied in the presence of 10% serum. However, the results described above would imply that, unlike FR3T3, they should grow in medium supplemented with 0.5% serum. This prediction is confirmed by the data shown in Table 6, strongly suggesting that expression of the N-terminal portion of the large T protein is sufficient to reduce the growth factor requirement of FR3T3 cells.

DNAs separately encoding the early proteins do not transform primary embryo fibroblasts

We have thus far described experiments with FR3T3 rat cells which demonstrate an effect of the large T gene on cellular growth regulation: expression of the large T protein, or only its N-terminal region, confers on both parental and middle T-transformed FR3T3 cells a reduced dependence on serum growth factors. To test the more general significance of our findings, we decided to investigate the requirements for transformation of non-clonal fibroblast cultures derived from primary explants of rat embryos ('primary' cells). Polyoma virus is known to be able to transform primary rodent cells. In addition to the transformation characters observed in transformed derivatives from established normal cell lines, it was recognized that virus transformation enables the cells to grow in culture for an unlimited period[30]. When plasmids containing wild-type or modified viral genomes were transferred into primary rat fibroblasts, transformation resulted only with the wild-type DNA (Table 2). Plasmid pPyMT1, encoding only the middle T protein and active in parallel experiments in the transformation of FR3T3 cells, did not transform primary cells, even in medium containing 10% serum. Experiments are in progress to determine whether the pairwise transfer of early genes would transform these cells.

Concluding remarks

We have confirmed previous evidence that the middle T protein alone can confer on established cell lines a phenotype similar to that of wild-type polyoma-transformed cells. Expression of this protein, however, was not sufficient to maintain the transformed state in low serum growth conditions in the highly serum factor-dependent line FR3T3. The low serum growth defect of FR3T3 lines expressing only the middle T protein could be complemented with equivalent efficiencies by DNA encoding either all or no more than the N-terminal 40% (334 amino acids) of the large T protein. Similarly, co-transfer of DNA encoding either the full-size large T protein or only its N-terminal region complemented the ability of the middle T gene to transform FR3T3 cells in low serum medium, yielding transformants which were not dependent on exogenous growth factors for expression of the transformed phenotype. Transfer of the full-size or truncated large T gene alone was sufficient to promote growth of normal FR3T3 cells at low serum concentrations, but did not induce expression of other properties (lack of topoinhibition, anchorage independence) characteristic of transformation. We therefore conclude that the middle T and large T gene products have independent effects on cellular physiology which together are responsible for the phenotype of wild-type transformants.

An important motivation for the collaborative efforts reported here was to resolve the apparent discrepancy between the observation that middle T expression alone was sufficient to transform established rat cells of the F2408 line[8] and reports that at least the N-terminal region of the large T gene was additionally essential to maintain the transformed state in lines derived from FR3T3 by infection with the *tsa* mutant of polyoma virus[21]. We found that cell lines derived from FR3T3 after transfer of the pPyMT1 plasmid conditionally express the transformed phenotype in a manner dependent on growth factor concentration. The same DNAs which were able to complement *tsa* transformants for transformation at the restrictive temperature were shown to complement the serum-dependent defect of cell lines expressing only middle T. Thus, the present experiments confirm the original suggestion[31] that the large T protein, or only its N-terminal region, plays an important part in the maintenance of transformation.

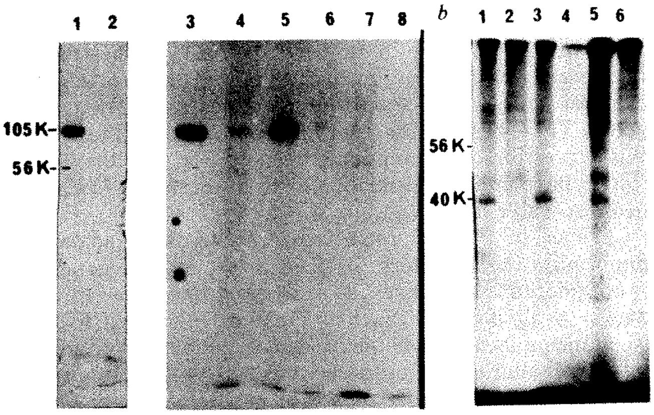

Fig. 3 Immunoprecipitation analysis of the viral proteins synthesized in cells derived from MTT transformants by complementation with the full-size or truncated large T genes. Cell lines MLT1 and MLT2 were independently derived from MTT4 cells after transfer of plasmid pPyLT1, and cell lines MMC1, MMC2 and MMC3, from MTT4 cells after transfer of plasmid pMC1 (see Table 1), by selection for colonies grown in agarose medium in the presence of 0.5% serum. Cells were subcloned twice in succession and routinely maintained in DMEM containing 0.5% serum. Before labelling, the cells were seeded at a density of 10^5 cells per 10-cm Petri plate and grown for 4 days in the presence of 10% serum. Labelling was for 2 h in the presence of 250 μCi of ^{32}P-orthophosphate (Amersham France) in 1 ml of Tris-buffered isotonic saline solution. Extracts were prepared and analysed by immunoprecipitation with anti-polyoma T antigen immune serum as described in ref. 21. Concentration of polyacrylamide was 10% for the gel shown in *a*, and 12.5% for that in *b*. The indicated electrophoretic mobilities corresponding to apparent molecular weights of 105,000, 56,000 and 40,000 were deduced from the position of protein markers on the same gels[21]. *a*: lane 1, MLT2 cells, immune serum; lane 2, MLT2 cells, control serum; lane 3, MLT1 cells, immune serum; lane 4, MLT1 cells, control serum; lane 5, PYT21 cells, immune serum; lane 6, PYT21 cells, control serum; lane 7, MTT4 cells, immune serum; lane 8, MTT4 cells, control serum. *b*: lane 1, MCC3 cells, immune serum; lane 2, MCC3 cells, control serum; lane 3, MCC2 cells, immune serum; lane 4, MCC2 cells, control serum; lane 5, MCC1 cells, immune serum; lane 6, MCC1 cells, control serum.

Table 5 Transformation frequencies after co-transfer into FR3T3 cells of polyoma sequences encoding different early proteins

Bacterial protoplasts carrying:	Transformants per 10^5 cells in DMEM supplemented with: 10% Calf serum Foci	Agar	0.5% Calf serum Foci
pBR322	0	0	0
pBR322 + pPY1	95	48	65
pBR322 + pNG1	0	0	0
pBR322 + pPyLT1	0	0	0
pBR322 + pMC1	0	0	0
pBR322 + pPyMT1	74	37	0
pBR322 + pPyST1	0	0	0
pPYMT1 + pNG1	79	16	31
pPYMT1 + pPyLT1	NT	15	39
pPYMT1 + pMC1	46	14	22
pPYMT1 + pPyST1	60	27	0*

FR3T3 cells (2×10^5 cells per 35-mm plate) were fused either with 2×10^9 protoplasts of *E. coli* 1106 carrying pBR322 or with the same total number of protoplasts prepared from mixed cultures (50:50) carrying the indicated plasmids. After 24 h, two plates from each group were trypsinized and the cells were seeded in four plates, two in liquid medium and two in agarose medium, in DMEM supplemented with 10% calf serum. Four days later, two more plates from each group were transferred into two 10-cm plates in medium supplemented with 0.5% serum. Foci and agar colonies were counted at day 16. Numbers are means of two plates (10% serum) or four plates (0.5% serum).

* Minute foci of slow-growing cells were observed after prolonged incubation of the plates (4 weeks).

We still, however, cannot explain why FR3T3 cells after transfer of the middle T gene have only a serum-dependent conditional defect in transformation, whereas *tsa* virus transformed cells of the N type revert to a normal phenotype at 40 °C in the presence of 10% serum[31]. We recently transferred into MTT FR3T3 cells a viral genome encoding only large T and carrying the *tsa* mutation (M.R. and F.C., unpublished experiments). These cells display a serum-dependent transformed phenotype at 40 °C but not at 33 °C. This result provides further important evidence for the continuous requirement for a large T gene product to maintain transformation in low serum medium, but differs from that obtained with *tsa*-transformed cells. The difference may result from the establishment of the transformed lines in recent experiments by DNA-mediated transfer of a large number of gene copies per cell, rather than by virus infection at low input multiplicities[31] in the former study. Also, the *tsa* transformants were established at the permissive temperature, in the presence of an active large T protein. A role for this protein in the formation of the head-to-tail tandemly integrated viral genomes commonly observed in virus transformants was indicated by recent experiments using *tsa* mutants[32]. Another approach is suggested by results obtained with temperature-dependent SV40 *tsa* transformants[33] indicating that these cells behave as transformed cells at 40 °C in the presence of 20% serum, but not with only 10%

Table 6 Derivation of cell lines with reduced serum requirement by selection in medium containing 0.5% calf serum after transfer into FR3T3 cells of large T coding genes

a, Plating efficiency of FR3T3 cells after transfer of plasmids encoding large T

Plasmid	No. of colonies 5×10^3 Cells per plate	 10^4 Cells per plate
pBR322	10	28
pPyLT1	80	150
pNG1	100	NT
pLT214	60	132
pMC1	89	160

b, Growth of FR3T3 cells and of FR3T3 derivatives selected after transfer of genes encoding large T

Cell line	Relative cell growth in DMEM containing: 10% Serum (day 8)	 0.5% Serum (day 8)	 (day 15)
FR3T3	60	1	1
PYT21	800	70	400
FR3T3–BR70	110	9	41
FR3T3–LT1	100	12	38
MTT4	800	14	40

a, FR3T3 cells were fused with protoplasts carrying the indicated plasmids as described in Table 2 legend. After 24 h, the cells were trypsinized and replated at the indicated densities in 10-cm Petri plates in medium supplemented with 0.5% serum. Giemsa staining and colony counting were done 1 month later. In these conditions, the efficiency of cloning of the FR3T3 cell line in DMEM containing 10% newborn calf serum is of the order of 50%. *b*, FR3T3–BR70 cell line was derived from FR3T3 cells after transfer of plasmid pMC1 (ref. 19, and see text) and FR3T3-LT1, after transfer of plasmid pPyLT1 by selection of clones in medium containing 0.5% serum (*a*). Cells were seeded at day 0 at a density of $1–5\times10^4$ cells per 60-mm Petri plate, depending on the line, in medium containing the indicated concentration of serum. Cells of parallel cultures were trypsinized and counted at days 0, 8 and 15. Values shown are ratios of cell number at the indicated time to that at day 0. Values underlined correspond to confluent arrested monolayers. These cultures resumed their growth after trypsinization and seeding at lower density in the same medium.

serum. Both polyoma *tsa* and MTT lines could thus in fact be serum-dependent transformants, the critical serum concentration being higher for the *tsa* lines than for MTT cells. Experiments are in progress to test these hypotheses.

Our present data suggest that the region of the large T protein involved in the alteration of cell growth control is localized within its amino-terminal 334 residues. This genetically defined domain would retain its biological activity after deletion of other parts of the polypeptide chain, as previously demonstrated for various multifunctional proteins, such as *Escherichia coli* DNA polymerase I[34]. Experiments using available mutants[35] are in progress to localize more precisely this functional domain. It may be encoded by the segment of genomic DNA with two open reading frames that also encodes the C-terminal half of the middle T protein (see Fig. 1). Deletions within this region of the genome are already known to affect tranformation[35]. Are the two functions of polyoma virus which cause oncogenic transformation encoded by alternative reading frames in a single DNA sequence?

We further show here that DNA encoding only the middle T protein does not stably transform rat embryo fibroblasts. We suspect that transformation of primary cells also requires a function exerted by the large T protein, that would confer on cells an unlimited growth potential in culture ('immortalization' function). Recent experiments demonstrated that transfer of DNA encoding only large T into primary rat embryo cells allows the establishment of permanent cell lines which do not express other transformation characters; these lines, as one might predict, could be efficiently transformed by plasmid pPyMT1 DNA (ref. 10 and M.R., Z. Naghashfar, N.G., R.K. and F.C., manuscript in preparation). This function of the large T gene may be related to its ability to reduce the serum requirement of established cell lines because it is also retained by the genes encoding only the N-terminal part of the protein.

Responsiveness to growth factors, modulated by the large T protein, may thus appear as critical both in the expression of oncogenicity and in the acquisition by primary cells of an unlimited growth potential in culture. Further biochemical investigations should define the nature of the growth factors involved. Recent experiments on cell lines expressing only the middle T protein (ref. 28 and B. Ozanne, personal communication) indicate that these cells secrete in the culture medium growth factor(s) that transiently induce the expression of a transformed phenotype in normal cells. Cells synthesizing the other viral polypeptides, including large T, secrete elevated levels of such factor(s) and are more responsive to growth factors. These observations may account for the fact that FR3T3 cells expressing only the middle T protein or only the large T protein have indistinguishable growth properties in low serum medium (Table 6).

Changes in the cell requirement for growth factors and immortalization in culture, induced by the large T gene, may be considered from our results as an early step in the transformation of rat fibroblasts. Such observations might help to establish at the molecular level the concept of neoplastic progression by 'graded alterations', proposed long ago by Rous (see ref. 36 for review) and originally applied to papova virus transformation by Vogt and Dulbecco[30].

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Making ends meet: a model for RNA splicing in fungal mitochondria

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On the basis of available nucleotide sequence and genetic data, we present a model for RNA splicing in fungal mitochondria. Seven intron RNAs of two fungal species can form identical secondary structures, involving four conserved sequences, which bring the ends of each intron together and allow an internal guide RNA sequence to pair with exon bases adjacent to the splice junctions. The splicing sites are thus aligned precisely within a conserved structure, which we suggest could present specific recognition signals to the proteins that catalyse the splicing reaction.

MANY eukaryotic genes are interrupted by DNA sequences which do not code for any part of the gene product. These DNA sequences are known as introns (or intervening sequences), whereas the coding regions of the gene are called exons. Introns have been found in eukaryotic mRNA, tRNA and rRNA nuclear genes, and also in mitochondrial mRNA and rRNA genes of fungi[1-3] and at least one plant mitochondrial mRNA gene[4]. In all cases introns are transcribed as part of the primary RNA transcript and are then excised by 'splicing'—a precise cleavage and ligation reaction which leaves the various exons joined together end to end in their order of synthesis. The mechanism of splicing has not been fully elucidated for any intron. We now present a model which shows how the RNA of one class of mitochondrial intron could take up a well defined secondary structure, allowing the precise and unambiguous alignment of the ends of exons flanking the intron ready for the cleavage and ligation reactions. We have used two lines of evidence in constructing the model. Comparison of the DNA sequences of four mitochondrial introns of *Aspergillus nidulans* which we recently determined (ref. 5 and R.B.W. *et al.*, in preparation) and five of *Saccharomyces cerevisiae*[6-8] highlights certain key conserved sections of intron sequence. When these are taken as the basis of the formation of base-paired regions of the RNA molecule, further conserved RNA–RNA pairing between non-conserved sequences is revealed, and quite remarkably all these introns can form very similar RNA secondary structures. Genetic evidence, provided largely by the work of Slonimski and co-workers[9-12], shows that particular short segments of the intron sequence distant from the splicing sites are important in *cis* for splicing; our model explains the phenotypes resulting from *cis*-acting mutations.

The introns studied are listed in Table 1 and the terminology used is described in Fig. 1 legend. The sequences that are highly conserved in introns of both species[5] are named P, Q, R and S; their sequences and locations are given in Table 1. Seven of the introns have the internal primary structure shown in Fig. 1[9,13]: a *cis*-acting region occupies the 5′ end of the intron (which in mRNA introns has an open reading frame), a central protein-coding region extends from just beyond conserved sequence R to the stop codon and there is a 3′ *cis*-acting region with no open reading frame. The protein-coding region of YC4 has been shown[9,13] to code for a protein essential for the excision of YC4 ('mRNA maturase')[11], and this is probably the case for several other introns. The general features of the model will

Table 1 Important sequences within mitochondrial introns

Intron	5′ Splice Site	Sequence IG *	E	P	Q	R	E′	S	3′ Splice Site
YC4	↑- 6-	AUGGCCU -32-	UGUGC -3-	AUGCUGGAAA-192-	AAUCAGCAGG-65-	UCAGAGACUACA- 1-	GCACA -1137-	AAGAUAUAGUCC- 21-	↑
YOX4	↑- 6-	GUGGCC -34-	UUUUUC -3-	AUGCUGGAAA- 78-	AAUCAGCAGG-50-	UCAGAGACUACA- 1-	GAAAAA - 740-	AAGAUAUAGUCC- 36-	↑
Y$_\omega$	↑- 3-	UUACCCCCUUGUCCC -64-	AUUGGGUG -2-	UUGCUUAGAU- 25-	AAUAAGCAGC-49-	UCAACGACUAGA-34-	CGCCCAAU-848-	AUGAUAUAGUCU- 61-	↑
YOX3	↑-1005-	UAAUAAGAUGCCG -28-	CAACAC -3-	AUGCGGGAAA-165-	AAUCCGCAGG-95-	UCAGAGACUACA- 1-	GUGUUG - 28-	AAGAUAUAGUCC- 29-	↑
NOX1	↑- 250-	AAUGCCG -27-	CUUCGC -3-	AUGCAUGGAA-102-	AAUAUGCAGG-39-	UCAGAGACUACA- 1-	GCGAGG 22-	AAGACAUAGUCC-362-	↑
NOX2	↑- 9-	AUAGAAAACUGCCGGC- 6-	UUUGGC -3-	AUACUGGAAA- 28-	AAUCAGUAGG-32-	UCAGAGACUAUA- 1-	GCCAAA 869-	AAAAUAUAGUCC-124-	↑
NOX3	↑- 1-	AAAAAUAUAGGCUUCU- 3-	CGUUGC -3-	AUGCUGGGAC- 69-	UAUCAGCAGG-19-	UCAGAGACUUUA- 1-	GCAACG - 851-	AAGAUAAAGUCC- 83-	↑
NC	↑- 4-	ACAGAUGAACC -26-	UCCUGC -3-	AUGCUGGAAA- 74-	AAUCAGCAGG-30-	UCAGAGACUACA- 1-	GCAGGA - 764-	AUGAUAUAGUCC- 76-	↑
YC3	↑- 2-	AUAUAGAGGAUCC -							↑
Consensus				7878667586 AUGCUGGAAA	7886687887 AAUCAGCAGG	888778888758 UCAGAGACUACA		867878788887 AAGAUAUAGUCC	

The nucleotide sequences and locations within nine fungal mitochondrial introns of regions that are important for RNA splicing, including the conserved sequences P, Q, R and S, are shown. The latter are named in their order of occurrence in the 5′ to 3′ direction within the introns. The E, E′ and IG sequences are described in the text. Numbers indicate distances in bases between particular sequences. The asterisk marks the conserved G base of the IGS. P and Q are the same as A and B, respectively, in ref. 5.

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be presented first, followed by a detailed consideration of the supporting evidence.

A model of mitochondrial RNA splicing

Figure 2 gives a diagrammatic representation of the steps in the splicing process, and Fig. 3 shows the actual secondary structure of the folded intron RNA. The model proposes three major stages in the excision of these introns.

(1) Intron RNA forms a specific secondary, and presumably tertiary, structure involving a number (usually nine) of conserved RNA–RNA interactions, with the result that the ends of the intron are brought close together. The RNA–RNA pairings involving the conserved sequences P, Q, R and S are among the most critical. The P and Q sequences are synthesized early, and their pairing P4 and the associated P5 (Fig. 3) are important in nucleating the secondary structure. The R–S pairing P7 and the associated P8 (Fig. 3) bring the 3′ end of the intron close to R, which is in turn brought close to the 5′ end of the intron by the pairing of the E and E′ sequences. Proteins may also be involved, binding at specific sites on the intron RNA either to stabilize secondary structure or to aid the formation of the precise alignment structure in stage 2. We regard the characteristic secondary structure formed by pairings P3 to P9 in Fig. 3 as the 'core structure' of these introns. The formation of the core structure is a prerequisite for the next stage.

(2) An internal guide sequence (IGS) located just 5′ to the E sequence (Figs 1, 3) pairs with short stretches of RNA sequence at the end of the upstream exon and the beginning of the downstream exon, acting as an adaptor molecule that brings the two splicing sites tightly together, separated only by the length of a phosphodiester bond. The IGS–upstream exon pairing is P1 in Fig. 3, the IGS–downstream exon pairing is P10. The P1 and P10 pairings involving IGS make up the precise alignment structure, details of which are given in Fig. 6 for nine introns. In all cases the splice sites are precisely aligned, and are in similar chemical environments at the ends of symmetrically placed helices. As shown in Fig. 2, this structure also ensures that exon–exon ligation is favoured, because when the RNA is cut at the exon–intron junction the exon ends are held in the double-stranded region, while the intron ends are free to diffuse away.

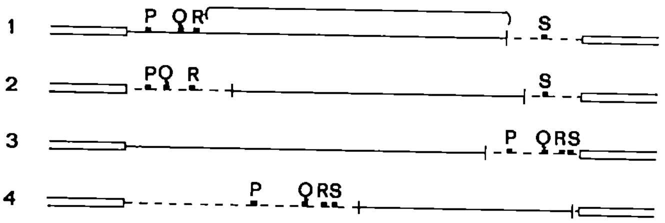

Fig. 1 A schematic representation of the internal primary structure of a class of fungal mitochondrial introns. Four different arrangements of open reading frames are shown; class 1 is the majority arrangement, found in six of the nine introns discussed here, while the other classes have one representative each. Internal features of each intron are shown to scale, but all introns have been scaled to the size of the class 1 intron. Symbols are as follows: open bars, exon sequences; continuous line, open reading frame within an intron; discontinuous line, intron sequence with no open reading frame. The bracketing line above the class 1 intron indicates the part of the yeast cob I4 intron in which *trans*-acting mutations of the *box*7 class have previously been located; this defines the maturase coding region. P, Q, R and S are the conserved sequences shown in Table 1. The results presented in this article are based on the comparison of nine intron DNA sequences, four from *A. nidulans* and five from yeast. We refer to these and other introns by species, gene and number only, symbolizing *S. cerevisiae* by Y and *A. nidulans* as N, and dropping 'intron'. Thus, yeast introns are written as follows: cob I3, YC3; cob I4, YC4; oxi3 I3, YOX3; oxi3 I4, YOX4; 21S rRNAω, Yω. *A. nidulans* introns are written as follows: cobA I1, NC; oxiA I1, NOX1; oxiA I2, NOX2; oxiA I3, NOX3. The data for Yω (ref. 8), YC4 (ref. 7), YOX3 and YOX4 (ref. 6) have been published, and those for NC, NOX1, NOX2 and NOX3 are from our laboratory and will be published in detail elsewhere (ref. 5 and R.B.W. *et al.*, in preparation). Nine introns NC, NOX1, NOX2, NOX3, YC3, YC4, YOX3, YOX4 and Yω, have been studied but we only show analyses based on eight of the introns plus those portions of the YC3 sequence that have already been published[21]. In this figure intron 1 = YC4 (YOX4, YC3, NC, NOX2 and NOX3 are similar to YC4), 2 = Yω, 3 = YOX3 and 4 = NOX1.

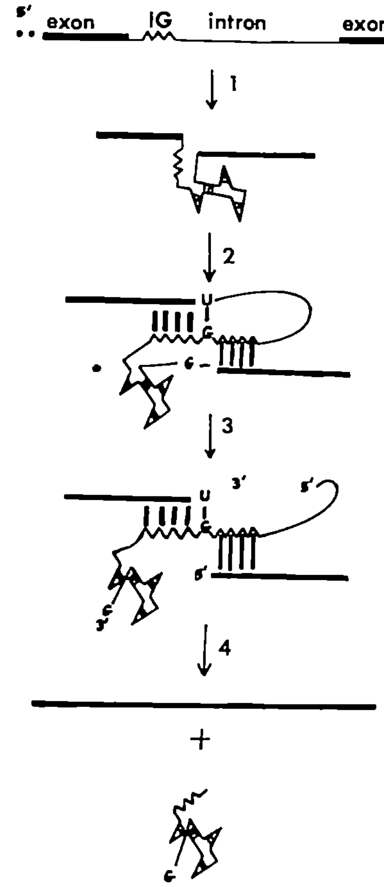

Fig. 2 Model of the role of the RNA substrate in mitochondrial RNA splicing. The model is described in the text. The steps shown here are: (1) formation of internal secondary structure; (2) formation of the precise alignment structure; (3) endonuclease binding followed by specific cuts to produce the ends shown and diffusion of free intron ends away from the splicing complex; (4) ligation of exon ends and release of linear intron RNA from the splicing complex. The precise alignment complex which is P1 + P10 of Fig. 3, is shown greatly enlarged between stages (2) and (4). The thick line represents exon RNA, the thin line intron RNA, the zigzag line the internal guide RNA sequence (IGS).

(3) The completed precise alignment structure is recognized by the splicing protein(s). The features recognized are the RNA structure *per se*, and certain chemical groups that only occur together near the splice sites in the complete structure, in particular the U · G base pair (bp) of P1 adjacent to the 5′ splice site and the G base at the 3′ end of the intron. In addition, the protein(s) recognizing this structure may also need to bind to components of the core structure or its associated proteins so as to bind efficiently. The specificity of the reaction is dictated by other non-conserved chemical signals and by the mRNA maturase[13]. Endonucleolytic scission at the 5′ and 3′ splice sites may be simultaneous or sequential. The free ends of intron RNA diffuse away immediately but the free ends of exon RNA are still held in the P1 and P10 pairings, and are ligated together by an RNA ligase which need not be specific. The splicing proteins are released from the IGS; the P1 and P10 pairings fall apart, releasing the linear intron (which is presumably subsequently destroyed) and the spliced mRNA.

The core structure

The fact that all introns considered here possess the same core secondary structure argues for the functional importance of this structure. This is strongly supported by our ability to explain the phenotype of all the sequenced *cis*-acting mutations affecting splicing, and by the maintenance of intact base-pairing despite variations from the consensus sequences. We stress that if any one intron RNA sequence is considered alone it is possible to find alternative ways of pairing the conserved sequences, but the model secondary structure presented in Fig. 3 is applicable to all the introns of both species. We have only considered pairings to be potentially biologically meaningful if they occur between identical or identically placed sequences of all the introns studied.

The extreme regularity and conservation of detail of RNA secondary structure within introns can be seen in Fig. 3*a*; all the conserved bases shown in the core structure in this figure are present in at least six out of eight introns and the conserved base pairs in all or seven out of eight introns. One of the most remarkable features that these introns have in common is the existence of a pair of sequences named by us E and E′ (Table 1, Fig. 3). Two bases after the conserved sequence R, there is a conserved G base in all the introns followed by a C base in five out of seven introns. These two bases together with the next four (three in YC4, five in YOX4 and Yω) make up the sequence we call E′. The amazing property of E′ is that, although the last four bases are completely different from intron to intron, they and the GC preceding them are always capable of pairing perfectly with a sequence E that precedes sequence P by three bases (Figs 3, 6). This conservation of precise base-pairing ability of sequences in identical positions in all introns, independent of actual nucleotide sequence conservation, demands the interpretation that these sequences really do pair.

Further evidence for the functional importance of the RNA–RNA interactions between P and Q, R and S is provided by looking at the effects on the pairings of variations from the consensus sequence. Sequences P and Q are strongly conserved between introns and species, but there are several variations from the consensus sequences. In all cases where a different nucleotide sequence is found within the section of P that can pair with Q, a compensatory alteration in sequence Q is found so that the base-pairing between the sequences is maintained. There are five such compensatory exchanges, the most striking being the case of NOX1, where an insertion of a base in P is exactly compensated for by two changes in Q. There are no alterations in the proposed base-pairing region of P that are not compensated for by complementary alterations in Q. The pairing regions of the R and S sequences are very precisely conserved; only in NOX3 does a change occur in part of the R sequence that pairs with S, and here too a compensatory alteration in S is found. All these compensatory exchanges in the variant sequences are shown in Fig. 4.

This model also accounts for the phenotypes due to several *cis*-acting mutations affecting splicing which have been sequenced by Slonimski and co-workers[9,12]. The mutations fall into two clusters, which in YC4 have been called *box*9 and *box*2. All the *box*9 mutations that have been sequenced (including the equivalent class in YOX4) fall in R or E′, and reduce the strength of base-pairing either of R and S or of E and E′; as R and E′ are separated by only one base, they have previously been considered to be part of one functional sequence, whereas we propose that they have different roles, although both are involved in secondary structure formation. Details of the sequenced mutations are given in refs 9 and 12, and their effects on the R–S and E–E′ interactions are shown in Fig. 5. Two mutations of the *box*2 class which affect sequence S of YC4 have also been sequenced[9]. Both W300-3 and G2590 alter G (ref. 8) to A; this would destroy a central G · C base pair in the pairing of S with R (Fig. 5). Thus, our proposal also explains the observed phenotype of *box*2 mutations. Weakening of the R–S or E–E′ pairings by these mutations will reduce the stability of the core structure, thus preventing efficient

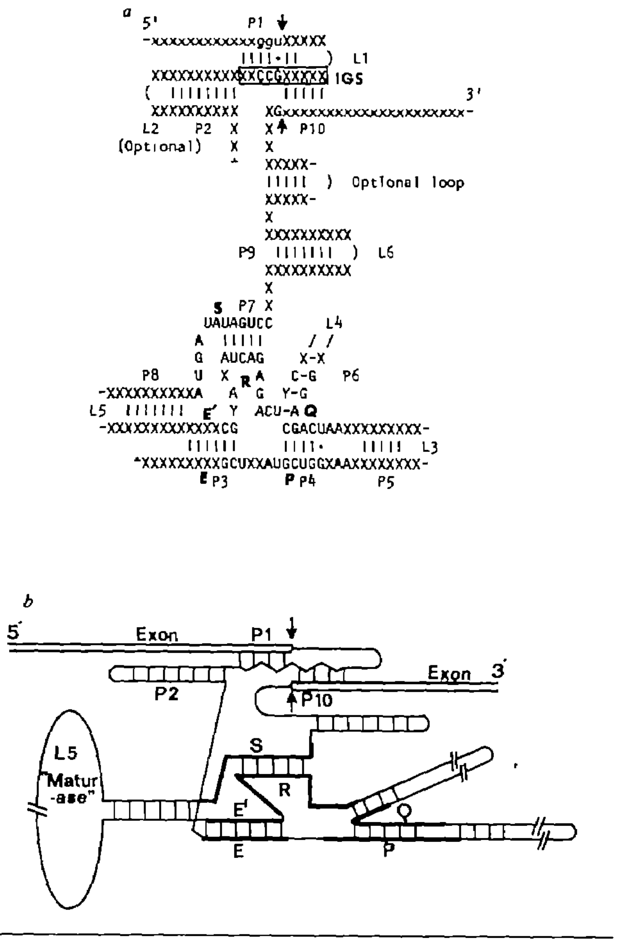

Fig. 3 Model RNA secondary structure for a class of fungal mitochondrial introns. *a*, A generalized secondary structure model derived from the RNA sequence of eight introns (YC3 data were not used), showing conserved bases and conserved pairings between non-conserved bases. *b*, A diagrammatic representation of the secondary structure showing only the key components. For convenience of two-dimensional representation, the RNA structure has been shown in *a* as if cut between P2 and P3 (or just 5′ to E)—the arbitrary ends that are left are marked with an asterisk and the sequence is continuous between these points. Intron bases are in capitals, exon bases in lower-case letters. A, U, C or G is written when these bases occur in a particular position in at least six out of eight introns, while Y indicates a pyrimidine and R a purine base in six out of eight introns; otherwise X or x signifies any base. Except for P1 and P10 (see Fig. 6) when a base pair is given, it occurs in at least seven out of eight introns in this position. When the end of a stem–loop structure is closed by a round bracket, the number of bases shown is that of the consensus intron; when the end is shown with a dash after each nucleotide, then a large variable number of bases is found in the loop (see Table 1). Base-paired regions are labelled P1 to P10, loops L1 to L6. L5 contains the maturase-coding region of the majority class intron; the putative maturase-coding region of YOX3 occurs in L1, and this loop is also large (though non-coding) in NOX1. YOX3 and NOX1 have small L5 loops, all others have small L1 loops of the average size shown in the consensus structure. P3 is formed by the pairing of P and Q, P7 by the pairing of R and S, P1 and P10 by the pairing of IGS with exon sequences. The central core of the structure is P3, P4 and P7 with the associated P5, P6 and P8. P2 and P9 are peripheral stems which bring the splice sites close to the folded central core; P1 and P10 form the precise alignment structure. In *b* exons are shown as open bars, conserved sequences as thick solid lines, IGS as a wavy line, and base pairs are shown where conserved. The line joining P2 to E is of necessity out of scale, and represents only four bases on average. The L5 loop is shown as coding for the maturase protein as in YC4, but it can code for non-maturase protein (Yω), be defective for protein coding (YOX4, NOX3), or very small (NOX1, NOX3).

formation of the precise alignment structure. This is why mutations within an intron cause splicing defects.

No mutations have so far been isolated in P, Q or E. This is probably because the genetic map is not saturated, that is, only a small proportion of all the possible mutations affecting splicing have been identified. Most of the mutations that have been isolated in R, S and E' are leaky[13], so if point mutations in P and Q had slightly weaker effects they could be difficult to detect. Alternatively, the mutations that have been isolated may affect not only RNA–RNA pairing but also alter the specific binding sites of proteins involved in splicing, and the P–Q pairing might not bind any protein at all. The model predicts that the distribution of small deletion mutations would be much more widespread and include P and Q, that point mutations will be found in E, and that E mutations will be found as suppressors of E' mutations and vice versa.

The precise-alignment structure and the internal guide sequence

At some stage of splicing the splice sites must be aligned precisely and held together long enough for the excision of the intron and the ligation of at least the exon ends to be achieved. Although this could conceivably be done by proteins alone, a very reliable and precise way of aligning two nucleotide sequences is by way of complementary base-pairing with an adaptor molecule[14,15]. We propose that this class of fungal mitochondrial intron RNAs possesses an internal guide sequence that functions as an adaptor, pairing with upstream and downstream exon sequences adjacent to the splice sites in such a way as to place the splice sites together exactly. The base-pairing in this precise-alignment structure need not be very extensive because we propose that the previous folding of the RNA core structure strongly favours the pairing of the internal guide sequence with the exon RNA sequences. In fact, most of the precise-alignment structures shown in Fig. 6 involve base-pairings (P1, 3–6 bp; P10, 3–8 bp) that are comparable with those proposed for the pairing of the U1 external guide RNA with intron sequences in the alignment of the ends of nuclear mRNA introns[14,15] without previous folding.

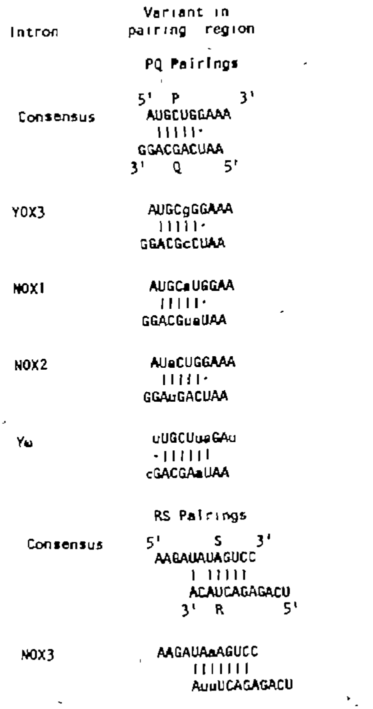

Fig. 4 The P–Q and R–S pairings with variants. Consensus pairings and all known exceptions are shown, demonstrating the conservation of base-pairing in the variant introns. Bases that differ from consensus are shown in lower-case, consensus bases in capitals. Intron terminology is given in Fig. 1 legend.

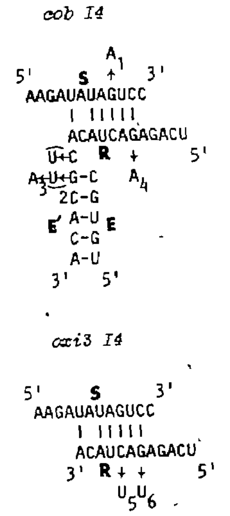

Fig. 5 Mutational evidence for the R–S and E–E' pairings. The R–S pairing of YOX4 and the R–S and E–E' pairings of YC4 are shown with all the sequenced *cis*-acting mutations affecting splicing[9,12]. Numbers next to bases in mutants indicate the identity of the mutant[9,12,13]: 1 = G2590 and W300-3; 2 = M4474 (a double base change); 3 = M7832; 4 = V353; 5 = G2190; 6 = G192.

The internal guide sequences are given in Table 1, and the IGS–exon pairings making up the precise-alignment structures are shown in Fig. 6; these correspond to P1 and P10 of Fig. 3. A similar though slightly shifted version of an alignment structure was proposed for Yω by Bos *et al.*[16]. If P1 is considered in isolation it involves an extra 1 or 2 bp beyond the U · G pair shown as the end of P1 in Fig. 6; these base-pairings are between the first bases of the intron and one or two of the IGS bases that can also take part in the P10 pairing.

The first argument in favour of this structure is the regularity with which it can be formed by a sequence (IGS) between the 5' splice site and sequence E. There are several conserved features, which can be located in Fig. 3 and seen in detail in Fig. 6. The finding that the precise-alignment structures are variable apart from these features agrees well with the considerable specificity of splicing reactions; note that the alignment structures and IGS of YOX4 and YC4, which share the same splicing specificity[13], are very similar.

The phenotypes resulting from two known mutations can be explained if the mutations reduce the strength of the pairings in the precise-alignment structure. G2457 is a mit$^-$ mutation of yeast that is clearly defective in the excision of YC4, but has been shown by DNA sequencing to be a single G → A transition affecting the last but one nucleotide of the upstream exon[9]; this G → A change would significantly reduce the stability of the pairing, as well as affecting the potential protein-binding site. This G and its pairing with a C in IGS within the P1 pairing are strongly conserved. The other example is the class of mutations known as ω^n, which occur in the gene for the large mitochondrial rRNA of *S. cerevisiae*; ω^n mutations remove the strong polarity (biased transmission of parental genotypes to offspring) that is otherwise found in crosses between ω^+ and ω^- strains of *S. cerevisiae*. ω^+ and ω^- strains differ in the presence and absence of the Yω intron, and ω^n mutations only occur in ω^- strains, never in ω^+ strains. ω^n mutations have been shown by DNA sequencing[8] to be a G → A transition three bases before the end of the upstream exon. This would reduce the strength of the P1 pairing in our model. Clearly, if this prevents RNA splicing, ω^n mutations could never occur in ω^+ strains because the Yω intron would not be excised and this would be lethal.

The proposed structure thus fulfils all the criteria for a structure having a critical role in determining the precision of the splicing reaction. As for P, Q and E, we expect mutations to be found in the IGS and further mutations to be found in the exon sequences near splice junctions as the genetic map becomes saturated.

Two introns which definitely have the core structure but only weak precise-alignment structures differ from the seven other introns in having extra DNA present between at least one splice junction and the core structure sequences (Fig. 1). The core structure of these introns is still necessary (hence its conservation) but not sufficient for bringing the ends of the intron together. There are two ways of interpreting the structure of NOX1 and YOX3. First, these introns are now spliced in a different way from the other seven, and either a completely new recognition mechanism has been superimposed or intron excision occurs in multiple steps, one of which may correspond to the standard model. Second, they are still spliced in the standard way, the extra DNA contributing additional secondary structure to correct for its own presence.

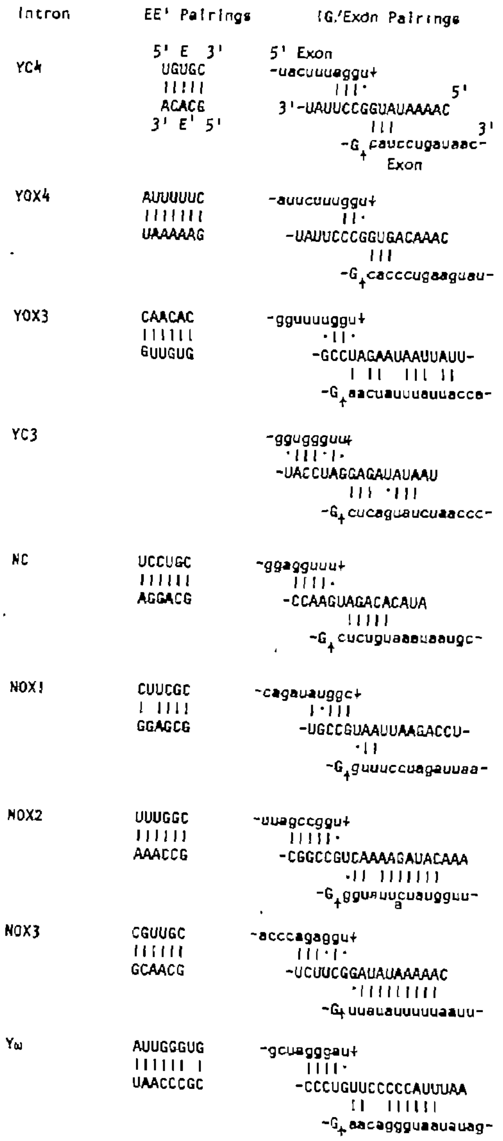

Fig. 6 Base pairing between the internal guide sequences and exon sequences adjacent to the 5′ and 3′ splice sites, and base pairing between E and E′ in nine fungal mitochondrial introns. Intron bases are in capitals, exon bases in lower-case lettering. The precise alignment structures are shown for convenience as if both the 5′ and 3′ cuts have been made. The intron bases that were in the L1 loop (Fig. 3*a*) are shown as a linear array in the middle line with the first (5′) base of the intron on the right, except where a dash indicates that a large number of bases are present 5′ to the sequence shown. Arrows indicate the cut sites. The last (3′) base of the intron is shown to the left of the 3′ cut site because this G is a conserved feature of the precise alignment structure. The one or two IGS bases that can pair either in P1 or P10 (Fig. 3*a*) are thus shown in their P10 pairing mode.

Proteins and the splicing reaction

The model deals with the role of RNA sequences in the splicing process, and not with the role of proteins or the mechanism of the reaction itself. It demonstrates, however, that proteins are probably involved in stabilizing RNA secondary structure as well as in the cutting and ligation steps. Splicing proteins may also need to recognize parts of the secondary structure instead of, or as well as, sequences near the splice junctions.

The form of the precise-alignment structure has consequences for the mechanism of the splicing reaction. IGS bases have two alternative pairings as shown in Fig. 3*a*. Once the core structure, perhaps aided by proteins, allows the P10 pairings to form, the two forms of the structure must exist in an equilibrium due to branch migration unless one is favoured by the local environment. If the fully formed P10 pairing is present for a reasonable fraction of the time, or is stabilized by proteins, then simultaneous cuts could be made at the 5′ and 3′ cut sites in a structure which has both the cut sites at the junction of a very short (half-turn) helical segment with single-stranded RNA, and separated only by the length of a phosphodiester bond.

It is important to make the two cuts spatially and temporally close together so as to reduce the error rate of the reaction, and this can be achieved if recognition and cutting of the 5′ and 3′ ends only occur efficiently once the whole structure has formed. Given this proviso, a sequential mechanism is possible with a nick first occurring at the 5′ scission site in the top strand of the full P1 pairing of Fig. 3*a*; the last base pair or two of P1 then dissociate or are displaced by P10 branch migration, and the 3′ cut is made. Even a fully sequential mechanism is possible if P1 is stabilized by proteins after the 5′ cut has been made. In certain nuclear mutant[17] and *cis*-acting mutant[18] strains of yeast, the 5′ cut can occasionally be made independently of the 3′ cut.

A strong body of evidence[13] supports the involvement of intron-coded mRNA maturases in the excision of some of these introns. These could be important at any stage in the splicing process, including the endonuclease reaction. One specific problem of mitochondrial RNA splicing is that RNA synthesis and splicing and protein synthesis occur in the same compartment in mitochondria, so that the RNA being spliced is simultaneously being translated. The hypothesis that ribosomes have a positive role in splicing[9,13] has received a little experimental support[19]. Ribosomes are likely to disrupt any secondary structure that does form, and in most of these introns ribosomes must cross the 5′ splice junctions to synthesize the maturase. A positive role for ribosomes within the framework of our model might be to inhibit premature 5′-end cutting by disrupting the P1 pairing, although there are alternative ways of achieving this. However, ribosomes travelling into the intron will tend to disrupt the core structure. We have assumed that ribosomes are sufficiently well spaced to allow the splicing complex to form (ribosomes within loops should have little effect). If ribosomes are closely spaced, one of the roles of proteins binding to the core structure may be to prevent the disruption of the structure by ribosomes. In this case it is possible that the maturase is involved in stabilizing the structure against disruption by ribosomes, as well as acting as at least a specificity factor in the splicing reaction.

Conclusions

The class of fungal mitochondrial introns which we have considered possess, according to our proposal, all the specific elements required for their own excision. The model presented here accounts for all the data at present pertaining to the precise excision of these introns, and makes clear predictions of classes of mutations that should be found as well as suggesting the possible types of activity of proteins involved in splicing. In this model there can also be no ambiguity in the splicing of multiple introns within one RNA precursor molecule, as the dependence of the formation of the splicing complex on previous folding of the intron RNA ensures that the 5′ and 3′ ends of the same intron are always chosen for the excision reaction.

This mechanism of RNA splicing may be of more general importance, because at least one nuclear intron, that in the gene for the large nuclear rRNA of *Tetrahymena*, fits this model precisely (R.B.W. *et al.*, in preparation).

We thank Drs J. Lazowska and P. P. Slonimski for communicating some of their data on YC3 before publication, and Dr B. Dujon for pointing out that Yω had certain features of this class of intron. We acknowledge the help of many workers in the field, but particularly Professor Slonimski and his group whose precise genetic analysis of mitochondrial RNA splicing provided essential evidence. This work was supported by MRC Hproject grant G7904290CB to R.W.D. and C.S.

Note added in proof: One prediction of the model has already been fulfilled: Dr P. S. Perlman and co-workers (personal communication) have obtained a mutation in E that blocks splicing of YC4. Dr R. Schweyen and co-workers (personal communication) have obtained the same base change in E as a revertant of a *box*9 mutation in E′, and the double mutant has regained an intact pairing region. Computer-aided analysis of intron RNAs of *S. cerevisiae* and *Kluyveromyces thermotolerans*[20] has provided RNA structures in excellent agreement with the consensus core structure presented here.

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Characterization of a regulatory region upstream of the *ADR2* locus of *S. cerevisiae*

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We have used in vitro *mutagenesis to identify a DNA sequence that is required for glucose regulation of the* ADR2 *locus of* Saccharomyces cerevisiae. *This region, located between 200 and 1,000 base pairs upstream of the structural gene for ADH II, has been identified by genetic and biochemical analysis as the site of* ADR3, *a* cis-*acting regulatory locus. If this region is deleted,* ADR2 *is no longer repressed by glucose. Moreover, if the sequence is excised and ligated in front of the* ADC1 *gene, it puts that gene under glucose control.*

THE eukaryotic promoter has been identified by site-specific mutagenesis and deletion analysis of cloned genes[1–6]. In several cases it has been possible to identify regions which have regulatory functions[7–10], and occasionally, DNA sequences immediately 5′ to the transcription initiation site have been implicated in regulating the expression of the adjacent structural gene. In other cases regulatory DNA sequences have been identified at a considerable distance from the affected gene[11–14].

We have chosen to examine the regulatory regions which affect the expression of the alcohol dehydrogenase (ADH) isozymes of the yeast, *Saccharomyces cerevisiae*. The 'classical' ADH I is synthesized during fermentative growth and is repressed about fivefold during growth on ethanol-containing medium[15]. A second isozyme, ADH II, is repressed when yeast are grown on glucose and is derepressed 100-fold when yeast are grown on a nonfermentable carbon source[16]. The structural genes coding for both ADH I (*ADC1*) and ADH II (*ADR2*) have been identified genetically[18], cloned[19,20] and their DNA sequences determined[21,22].

We show here that deletion of a specific 5′-flanking sequence of *ADR2* results in the loss of glucose control of ADH II synthesis. This sequence overlaps *ADR3*, a genetically defined *cis*-acting locus which regulates *ADR2*[23]. In addition, we show that these sequences can be removed from their normal context in front of *ADR2*, placed upstream from the promoter of a different gene (*ADC1* in this case), and still retain their function.

Construction of an *ADR2* deletion

There is extensive nucleotide sequence homology in the region between the TATAA boxes and the transcription initiation sites of *ADC1* and *ADR2*[22]. The presence of an *Sph*I restriction site immediately 5′ to this homologous region in *ADR2* allowed us to test the hypothesis that regulatory sequences would be 5′ to the *ADR2* TATAA box. If this idea were correct, a deletion of sequences 5′ to the *Sph*I site would prevent glucose repression of *ADR2*. To minimize gene dosage effects, the *ADR2* gene constructs were inserted into pBC3T1, a stable, single-copy vector containing a yeast centromere from chromosome 3 (*CEN3*)[24] and the *TRP1* gene[17] (see Fig. 1). A 2.6-kilobase (kb) *Bam*HI/*Sph*I fragment containing *ADR2* and 1.2 kb of 5′-flanking sequence was cloned into pBC3T1 and designated pADR2. A second DNA fragment containing *ADR2* and only 176 base pairs (bp) of 5′ sequence was inserted into the *Sph*I site of pBC3T1. Clones with the fragment inserted in both orientations [pADR2(−176)R and pADR2(−176)L] were isolated.

These plasmids were used to transform the trp$^-$ yeast strain 302-21 which contains defective alleles of all three ADH structural genes: *adc1-11*, *adr2-43* and *adm* (the last gene codes for a minor ADH isozyme found in the mitochondria). Single TRP$^+$ colonies were isolated and grown on medium containing either glucose or ethanol. Cell extracts were prepared and assayed for ADH enzyme activity. As the results given in Table

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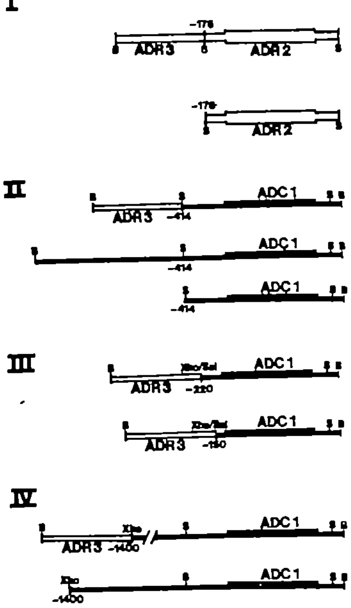
Table 1 ADH enzyme activities of transformed yeast cells



	Plasmid	ADH activity (mU per mg) Glucose	ADH activity (mU per mg) Ethanol
I	pADR2	30	1600
	pADR2(-176)L	1200	2000
	pADR2(-176)R	1100	4100
II	pADR3/ADC1(-414)	10	300
	pADC1(-2000)	2200	1300
	pADC1(-414)	4300	8300
III	pADR3/ADC1(-220)	10	200
	pADR3/ADC1(-150)	30	900
IV	pADR3/ADC1(-1400)	2000	1300
	pADC1(-1400)	2300	1100

The trp$^-$ yeast strain 302-21 was transformed with plasmid DNA according to the method of Beggs[29]. TRP$^+$ colonies were selected at random and assayed for enzyme activity in repression and derepression conditions using the following protocol. Cells from a stationary culture were inoculated into 10 ml trp$^-$ minimal media containing 8% glucose at a concentration of $\sim 5\times10^5$ cells per ml. These cultures were grown overnight at 30 °C to a concentration of $\sim 2\times10^7$ cells per ml. One half of the culture was collected and assayed, the remaining half was washed once in H_2O and resuspended in 5 ml trp$^-$ minimal media containing 3% ethanol. These cultures were grown for another 24 h at 30 °C and then collected. Assays were performed according to the method of Lutsdorf and Megnet[16]. Each value represents the average of the enzyme activities measured for three to five independent transformants.

1, section I, show, enzyme activity in strain 302-21 transformed with pADR2 is repressed on glucose-containing medium and derepressed on ethanol-containing medium. The amount of enzyme activity present in derepressed cells is comparable to that found for a strain containing a functional chromosomal *ADR2* gene (Table 2, strain 43-2B). Transformants of strain 302-21 containing an *ADR2* gene deleted to 176 bp 5′ to the structural gene [pADR2(−176)L and pADR2(−176)R] have high levels of ADH (>1,000 mU per mg protein) even on glucose-containing medium. This is true for the fragment cloned in either orientation with respect to the vector, thus arguing against the likelihood that the expression observed is due to transcription initiated at a vector site with readthrough of the *ADR2* gene. This result strongly implies that the region that has been deleted in the plasmids pADR2(−176)R and pADR2(−176)L (that is, the region 5′ to an *Sph*I site 176 bp upstream of the structural gene) contains sequences which are required for catabolite repression of *ADR2*.

Construction of an *ADR3/ADC1* hybrid

The identification of an *Sph*I site at −414 bp in the 5′-flanking region of *ADC1* allowed a further test of the hypothesis that the 5′ *Bam*HI/*Sph*I fragment of *ADR2*, which we will designate *ADR3*, contains regulatory sequences. If this region is essential for catabolite repression, ligation of the *Bam*HI/*Sph*I fragment in front of *ADC1* should confer glucose repressibility on ADH I. The *Bam*HI/*Sph*I fragment was ligated to an *Sph*I/*Bam*HI fragment containing *ADC1*, and the hybrid was cloned into the *Bam*HI site of pBC3T1 and designated pADR3/ADC1(−414). To compare the expression of this hybrid gene with that of *ADC1* containing its own 5′-flanking sequences, two other plasmids were also constructed. A 3.5-kb fragment containing *ADC1* and 2,000 bp of 5′-flanking sequence was cloned into pBC3T1, and designated pADC1(−2000). An *Sph*I fragment containing *ADC1* and only 414 bp of 5′-flanking sequence was cloned in the same vector. This construction, pADC1(−414), was tested to ensure that a deletion of sequences 5′ to −414 bp (the *Sph*I site) did not affect ADH I synthesis.

ADH activity was measured in cell extracts prepared from strain 302-21 transformed with the plasmids just described. Insertion of the *ADR3* sequence 5′ to the *ADC1* gene [pADR3/ADC1(−414)] dramatically reduces ADH activity when cells containing the plasmid are grown on glucose-containing medium (Table 1, section II). The enzyme activity increases after growth in derepressing conditions to about 20% of the value obtained in extracts of cells transformed with pADR2. Strain 302-21 transformed with either pADC1(−2000) or pADC1(−414) has abundant enzyme activity when grown on either glucose- or ethanol-containing medium. The enzyme activities found for pADC1(−2000) are comparable to values found in strains with a functional chromosomal *ADC1* gene. These results show that the repression observed with the hybrid gene, pADR3/ADC1(−414), is due to the *Bam*HI/*Sph*I fragment normally flanking *ADR2*.

To identify which ADH isozyme is expressed in these transformed strains, cell lysates were analysed by electrophoresis on a non-denaturing polyacrylamide gel and stained for ADH activity (Fig. 2). Only ADH II is detected in cell extracts prepared from yeast transformed with pADR2 and the plasmids containing deletions 5′ to ADR2. However, the isozyme present in cell extracts prepared from the strain transformed with the hybrid pADR3/ADC1(−414) is the *ADC1* gene product, ADH I. This proves that the repression and derepression of ADactivity that occur in cells transformed with the hybrid plasmid is not due to the inadvertent isolation of a transformed strain containing wild-type *ADR2*.

Construction of hybrids using *ADC1* 5′ deletions

Derepression of *ADC1* in the hybrid construction pADR3/pADC1(−414) is weak compared with that observed for pADR2. The efficiency of derepression might be influenced by the distance of the *ADR3* sequence from the promoter.

Table 2 ADH expression in strains containing regulatory mutations that affect *ADR2*

	ADH activity (mU per mg)							
	Strain 526-3 (*ADR2 ccr1*)		302-21 (*adr2 ADR1*)		SF6-4A-M4 (*adr2 ADR1-5^c*)		500-16 (*ADR2 adr1*)	
Plasmid	Glc	EtOH	Glc	EtOH	Glc	EtOH	Glc	EtOH
pADR2	20	47	30	1,600	360	5,600	47	1,100
pADR3/ADC1(−414)	NT	NT	10	300	43	1,400	30	2,500
pADR3/ADC1(−150)	9	15	30	900	170	3,100	8	1,300
pBC3T1 (vector)	1	10	3	11	7	10	2	16
	Strain: 526-3 (*ADR2 ccr1*)		43-2B (*ADR2 ADR1*)		R234 (*ADR2 ADR1-5^c*)		500-16 (*ADR2 adr1*)	
	Glc	EtOH	Glc	EtOH	Glc	EtOH	Glc	EtOH
Genomic *ADR2*	1	10	15	1,850	414	5.400	1	7

The enzyme activities of strains 43-2B and R234 are according to Ciriacy[25] All other activities were measured as described in Table 1 legend. NT, not tested.

Therefore, *ADC1* clones with deletions 3′ to the *Sph*I site were constructed and tested for their ability to express ADH I. Compared with a cloned *ADC1* gene with 2,000 bp of 5′-upstream sequence [pADC1(−2000)], deletion of 5′-flanking sequence to within 150 bp of the beginning of the structural gene has no effect on the level of *ADC1* expression when yeast containing these plasmids are grown on glucose-containing medium (results not shown).

The result that deletions of yeast DNA between 414 and 150 bp 5′ to the *ADC1* gene did not reduce *ADC1* expression enabled us to test the hypothesis that *ADR3* function in a hybrid construction is sensitive to its position relative to the *ADC1* promoter. The *Bam*HI/*Sph*I fragment (*ADR3*) was ligated closer to *ADC1* using the 5′-deleted plasmids pADC1(−220) and pADC1(−150), which are deleted to within 220 and 150 bp 5′ to *ADC1*, respectively. These hybrid gene constructions are designated pADR3/ADC1(−220) and pADR3/ADC1(−150).

These plasmids were used to transform strain 302-21; the transformed colonies were tested for enzyme activity and the results are shown in Table 1, section III. Ligation of the *ADR3* sequences 100 bp 5′ to the TATAA homology region (pADR3/ADC1(−220)) resulted in no increase of derepressed enzyme activity relative to the original hybrid construction. However, strain 302-21 transformed with pADR3/ADC1(−150), in which this sequence is only 30 bp 5′ to the TATAA homology region, has threefold higher ADH activity. The enzyme activity is ~60% of that found in the strain containing the cloned *ADR2* gene, pADR2.

These results demonstrate that the efficacy of the *ADR2* operator, *ADR3*, for the promotion of transcription of the adjacent gene during derepression depends on its placement with respect to the promoter of that gene. To test this idea further, a hybrid gene was constructed such that the *ADR3* region was located far upstream (~1,300 bp) from the TATAA sequence of *ADC1*. This plasmid [pADR3/ADC1(−1400)] and a control plasmid containing only *ADC1* sequence [pADC1(−1400)] were used to transform strain 302-21.

The enzyme activities of cell extracts of tranformants are shown in Table 1, section IV. The activities found for yeast containing either pADC1(−1400) or pADR3/ADC1(−1400) are nearly identical to those found for yeast containing pADC1(−2000) when the cells are grown in either repression or derepression conditions. It seems that the *ADR3* sequence exerts no effect on *ADC1* expression when it is ligated at a site 1,300 bp upstream from the *ADC1* promoter. However, it is possible that sequences upstream of *ADC1* in this construction interfere with repression.

Glucose control of *ADR2* and *ADR3/ADC1* in mutant backgrounds

Strain 526-3 (*adc1-11, ADR1, ADR2, ccr1*) fails to express ADH II enzyme activity when grown on ethanol-containing medium due to a mutation at the regulatory locus, *CCR1* (ref. 25). This strain was transformed with the plasmids pADR2 and pADR3/ADC1(−150), and the ADH activity in the transformants measured (Table 2). Extracts of strain 526-3 containing these plasmids did not contain significant ADH activity when cells were grown on ethanol-containing medium. Therefore, *CCR1* function is required for derepression of enzyme synthesis for both pADR2 and pADR3/ADC1(−150).

ADR1 is another unlinked regulatory locus which affects *ADR2* expression. The semi-dominant *ADR1-5*^{c} mutation has two effects: it results in partially constitutive ADH II synthesis, and the ADH II activity is increased to a level higher than is found in *ADR1* wild-type cells during derepression[25]. A strain containing the *ADR1-5*^{c} mutation, SF6-4A-M4 (*adc1-11, adr2-43, adm*), was transformed with pADR2 and hybrids pADR3/ADC1(−414) and pADR3/ADC1(−150). The enzyme activities of extracts from the transformed strains are given in Table 2. The enzyme activities shown for pADR2 correspond to those found in a strain containing a functional chromosomal copy of *ADR2* (Table 2, strain R234). The enzyme activities of cells transformed with the hybrid plasmids pADR3/ADC(−414) and pADR3/ADC1(−150) are significantly elevated for both repressing and derepressing growth conditions relative to the levels found in a host strain containing wild-type *ADR1*. Again, the enzyme activities found in yeast containing pADR3/ADC1(−150) are higher than in

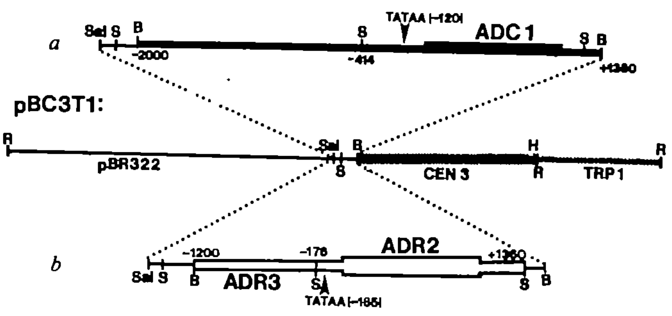


Fig. 1 Restriction map of vector pBC3T1 and yeast sequence inserts for pADC1(−2000) (*a*) and pADR2 (*b*). The vector pBC3T1 was constructed by first ligating a *Hin*dIII/*Bam*HI fragment containing the yeast centromere *CEN3*[24] into the *Hin*dIII and *Bam*HI sites of pBR322. Restriction enzymes and T4 DNA ligase (BRL) were used according to manufacturers' instructions Methods for preparative isolation of DNA fragments have been described elsewhere[20]. Plasmids from *E. coli* transformed to ampicillin resistance were analysed after extraction according to a method described by Holmes and Quigly[30]. The yeast *TRP1* gene was isolated from the yeast–*Escherichia coli* shuttle vector YRp7 by *Eco*RI digestion and ligated into the *Eco*RI site of the *CEN3*-pBR322 vector after treatment of the *Eco*RI-cut plasmid with calf intestine alkaline phosphatase (Boehringer). The orientation of the *TRP1* gene in pBC3T1 is the opposite of its orientation with respect to pBR322 sequences in YRp7. Plasmid pADR2 was constructed using a *Sal*I/*Sph*I fragment from YRp7–ADR2–BSb which contains a 2.7-kb insert of yeast sequences containing the *ADR2* structural gene[20]. This plasmid was digested with *Sal*I and partially with *Sph*I. A fragment containing 2.6 bp of yeast sequence including *ADR2* and 1,200 bp of 5′-flanking sequence was ligated into PBC3T1 cut with *Sal*I and *Sph*I. This construction has a direct repeat of ~200 bp of pBR322 sequences, which flank the yeast insert Plasmid pADC1(−2000) was constructed by isolating a 3.5-kb *Bam*HI fragment containing *ADC1* and 2,000 bp of 5′-flanking sequence from YRp9T6[19]. This was inserted into the *Bam*HI site of pBC3T1. To construct pADR2(−176)L, in which yeast sequences 5′ to the *ADR2 Sph*I site at −176 are deleted, plasmid pADR2(−1700) was partially digested with *Sph*I and religated. Plasmids in which the upstream sequences were deleted were identified by restriction digestion. To prepare pADR2(−176)R, which is a plasmid containing a deleted *ADR2* fragment cloned in the opposite orientation as pADR2(−176)L, an *Sph*I fragment containing the *ADR2* gene and 176 bp of 5′-flanking sequence was isolated. This fragment was cloned into pBC3T1 that had been cut with *Sph*I and treated with calf intestine alkaline phosphatase. The orientation of the inserted fragment was determined by restriction enzyme analysis. The plasmic pADC1(−414), in which the sequences 5′ to the *Sph*I site at −414 are deleted, was constructed in a similar manner to that described for pADR2(−176)L. A series of hybrid genes were constructed in which the *ADR3* regulatory region was ligated to a variety of sites 5′ to the *ADC1* gene and cloned in pBC3T1. Plasmid pADC(−414), with a deletion to the *Sph*I site at −414 of *ADC1*, was reconstructed to form a hybrid gene (pADR3/ADC1(−414)) by replacing the *Bam*HI/*Sph*I fragment upstream of *ADC1* with the 1-kb *Bam*HI/*Sph*I *ADR3* regulatory region. The plasmid pADR3/ADC1(−150) was constructed using an *ADC1* gene isolated from pADC1(−150), which contains a *Sal*I site ~150 bp 5′ to the *ADC1* structural gene. An *ADR3* fragment was prepared from a construction in which the *Sph*I site had been altered to a *Xho*I site using molecular recombination linkers (Collaborative Research). The 1-kb *Bam*HI/*Xho*I *ADR3* region and the *Sal*I/*Bam*HI *ADC1* fragment were ligated into pBC3T1 cut with *Bam*HI and treated with calf intestine alkaline phosphatase. Isolates in which the orientation of the structural gene with respect to the vector was the same as in pADC1(−2000) were identified by restriction analysis. The hybrid-gene plasmid pADR3/ADC1(−220) was constructed in the same manner, using *Sal*I/*Bam*HI fragment from pADC1(−220) in which a *Sal*I site is 220 bp 5′ to *ADC1*. The same method was used for the construction of pADR3/ADC1(−1400) using an *ADC1* fragment from a construction in which a *Hin*dII site, 1,400 bp 5′ to the structural gene had been converted to a *Xho*I site using molecular recombination linkers. This fragment was also used to construct pADC1(−1400), by cloning it into pBC3T1 cut with *Sal*I and *Bam*HI. Restriction enzyme cleavage sites are *Bam*HI (B), *Sph*I (S), *Sal*I (Sal), *Eco*RI (R) and *Hin*dIII (H).

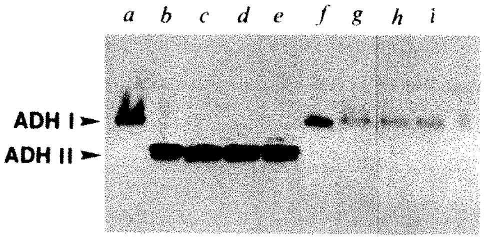


Fig. 2 Identification of the ADH isozyme expressed in transformed yeast cells. Cell extracts were electrophoresed on a non-denaturing polyacrylamide gel as previously described[19], and stained for ADH activity[28]. Lane *a* contains an extract from strain SF4-1B, which expresses only ADH I. Lane *b* contains an extract from strain R112, which expresses only ADH II. Lanes *c–i* contain extracts of strain 302-21 transformed with the following plasmids: *c*, pADR2; *d*, pADR2(−176)R; *e*, pADR2(−176)L; *f*, pADC1(−2000); *g*, pADR3/ADC1(−414); *h*, pADR3/ADC1(−220); *i*, pADR3/ADC1(−150).

yeast containing pADR3/ADC1(−414), in which the *ADR3* region is displaced further upstream relative to the *ADC1* promoter.

The recessive *adr1-1* mutation results in a very low level of *ADR2* derepression when cells are grown on ethanol-containing medium[23]. The plasmids described above were used to transform strain 500-16, which is defective for *ADR1 (adr1-1, adc1-11, ADR2, adm)*. Table 2 gives the ADH activities of these transformed strains and reveals the surprising result that the *ADR2* gene on a plasmid is abundantly expressed in derepressing conditions despite being in a host strain in which the chromosomal copy of *ADR2* is not expressed due to the *ADR1* defect. The hybrid pADR3/ADC1 plasmids also express ADH activity in abundance. In this case, pADR3/ADC1(−414) is more fully derepressed than in a wild-type *ADR1* background.

Analysis of cell extracts by electrophoresis detected only ADH I activity in the pADR3/ADC1 transformants, proving that the chromosomal copy of *ADR2* is not derepressed concomitantly with the plasmid genes. Independence of *ADR1* is not due to the absence of upstream control sequences in pADR2, as three multi-copy plasmids containing the *ADR2* gene with 1, 6 and 13 kb of 5′-flanking sequence are all derepressed in a strain containing the defective *adr1-1* allele (results not shown).

Discussion

Two different lines of evidence suggest that the promoter for transcription of *ADR2* can be separated from its operator region, *ADR3*. First, deletion of yeast sequences upstream from an *Sph*I site 176 bp 5′ to the coding region of *ADR2* allows constitutive synthesis of ADH II on glucose. Second, when these upstream sequences are ligated to *ADC1*, this gene becomes glucose repressible. Because the presumed TATAA box for *ADR2* transcription is located 160 bp 5′ to the coding region of *ADR2*, we conclude that all the sequences essential for efficient transcription of *ADR2* are located between the TATAA box and the initiation site of transcription, which is 54 bp 5′ to the coding region of *ADR2*.

The operator region, defined as the DNA sequences which can confer glucose repressibility on either *ADR2* or *ADC1*, extends upstream from approximately position −176 for not more than 1 kb. This same region overlaps the *ADR3* locus which was identified by *cis*-acting regulatory mutations that allow partially constitutive ADH II synthesis on glucose medium[23]. Most of these *ADR3*^{c} mutations are caused by Ty insertions between positions −210 and −125[26]. Two non-Ty induced *ADR3*^{c} mutations have been located by cloning and DNA sequence analysis at position −220 (D. Russell, personal communication). Thus, there is a good correspondence between the genetic locus, *ADR3*, and the DNA fragment that confers glucose repressibility.

The function of the *ADR3* region depends on its position with respect to the promoter of the adjacent gene. Glucose repression mediated by *ADR3* is effective when the fragment is as far as 300 bp upstream from the TATAA box of *ADC1*. In contrast, efficient derepression of the hybrid gene occurs only when *ADR3* is ligated within 30 bp of the promoter. The evidence that hybrid genes can be constructed in which derepression of *ADC1* is incomplete but repression is apparently normal suggests that repression and derepression might occur by independent mechanisms.

The repression and derepression conferred by the *ADR3* locus contained on plasmids is subject to some of the same regulation as is the chromosomal *ADR3* region. Derepression of the hybrid gene pADR3/ADC1(−150) requires *CCR1* function. *CCR1* is a pleiotropic effector which is required for the derepression of several glucose-repressed enzymes, including *ADR2*[25]. The hybrid gene also shows appropriate response (that is, increased enzyme activity) to *ADR1-5*^{c}, a regulatory mutation that results in partially constitutive synthesis of ADH II[25]. However, plasmids containing *ADR3* do not require *ADR1* for expression of the neighbouring structural gene (either *ADC1* or *ADR2*) as does the chromosomal *ADR2* locus. This result is not understood.

The identification of glucose control sequences for *ADR2* and the effect of operator–promoter separation on gene activity may help explain how transposable elements alter *ADR2* expression. The ADH II-constitutive phenotype resulting from Ty insertion may be a consequence of the displacement of the operator far enough upstream of the promoter to negate its effect, as proposed previously[26,27]. This hypothesis is consistent with the failure of *ADR3* to show any control over *ADC1* when it is ligated 1,300 bp upstream from its promoter, as in pADR3/ADC1(−1400). Secondary mutants of Ty-insertion *ADR2* mutants in which the Ty element has been replaced by a single 340-bp delta sequence have negligible ADH II activity when grown on glucose-containing medium and are only weakly derepressed[27]. In these mutants the position of *ADR3* with respect to the TATAA sequence is similar to that found in the hybrid pADR3/ADC1(−414). Cells transformed with this hybrid also have enzyme activities that are low when grown in either repression or derepression conditions.

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LETTERS TO NATURE

A new class of radio pulsars

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Sufficiently low magnetic field neutron stars which accrete for long times from a surrounding keplerian disk can be spun up to millisecond periods. After accretion ceases, such stars could become isolated or binary pulsars, depending on whether their companions have subsequently disrupted the binary by either their tidal break ups or their supernova explosions. Such pulsars constitute a new class, with short periods, long apparent ages ($P/\dot{P} \gtrsim 10^8$ yr) and pulsed optical, X-ray and γ-ray fluxes significantly below those expected for canonical pulsars with similar periods. Because of their long subsequent spin-down lifetimes, such pulsars would probably be observable if their birth rate exceeded even 10^{-4} that of the canonical ones. We propose here that the recently discovered millisecond pulsar 4C21.53 belongs to this new class, together with the binaries PSRs 1913+16, 0820+02 and 0655+64, as well as possibly several isolated pulsars such as PSRs 1952+29 and 1804−08. The possibility of such a class of fast accretion-spun-up pulsars has also been noted by Backus *et al.*[1].

Several kinds of observations support the view that many neutron stars in the close accreting binaries which constitute the galactic bulge X-ray sources have weak ($B \ll 10^{11}$ G) surface magnetic fields: (1) analyses of post-burst cooling of X-ray bursters imply that accretion has been effective over the entire neutron star surface with no important effects from magnetic funnelling to polar regions[2–5]; (2) in such neutron stars there is no sign of the modulation of X-ray emission expected from rotation of a magnetized stellar surface with $B \gg 10^{10}$ G (ref. 6); (3) good agreement with theoretical models for burst properties is achieved when magnetic funnelling is not effective ($B < 3\times10^{11}$ G)[7]. In addition, various scenarios which would be expected occasionally to lead to such binaries imply some very low field neutron stars[8]. Thus, for an accreting 'non-magnetic' white dwarf[9] ($B < 10^5$ G) which grows beyond the Chandrasekhar limit and collapses to a neutron star, simple flux conservation alone would be expected to amplify B to at most 10^9 G. Finally, theoretical models offer various reasons for expecting strong reductions in (dipolar) magnetic fields in many or most old ($\gg 10^7$ yr) neutron stars which cause them to be extinguished as pulsars[10,11].

There are a variety of scenarios for systems in which a neutron star accretes from a keplerian accretion disk initially fed by a companion[8,12]. First, low mass X-ray binaries (galactic bulge sources). Typical accretion rates are 10^{-9}–$10^{-10}\,M_\odot\,\text{yr}^{-1}$ for 10^9–10^{10} yr so that a mass $\gtrsim 10^{-1} M_\odot$ is transferred to the neutron star. Because of orbit shrinking due to gravitational radiation[12] the ultimate fate of such binaries must be a single neutron star or black hole. The low mass primary, even if it became a white dwarf first, will ultimately overflow its diminishing Roche lobe and be partly accreted onto the neutron star with the remainder dispersed in space. In some cases the accreting neutron star may become massive enough to collapse to a black hole, but often the remainder should be an isolated neutron star, embedded in the dilute remnant cloud of that part of the companion which was not accreted. For circular low mass binaries this last stage of evolution will take $<10^{10}$ yr if the initial binary period is of the order of 5 h or less[13]. For highly elliptical orbits such as that of the binary pulsar PSR 1913+16 this can be accomplished with longer initial periods. (We note that even a double neutron star binary such as PSR 1913+16 must ultimately end as a single rapidly spinning neutron star or black hole.)

Second, high mass X-ray binaries where mass transfer is very rapid ($\sim 10^4$ yr) into the Roche lobe of the neutron star. Much of this mass may ultimately form a keplerian disk before accretion onto the neutron star. The primary may then evolve to a supernova which disrupts the binary or leaves behind a bound collapsed companion. Neutron star accretion from a relatively massive partially filled Roche lobe may well continue long after the primary has disappeared if the Eddington limit rather than disk viscosity is controlling the accretion.

When the accretion region nearest to the neutron star is a keplerian disk the star will spin up until the keplerian angular velocity at the disk inner edge (the stellar magnetosphere's Alfvén radius) approximately equals that of the star. When enough matter is accreted to achieve this steady state, the equilibrium period[12,14,15] is

$$P \sim (6\times10^{-4}\ \text{s}) B_8^{6/7} R_6^{18/7} (M/M_\odot)^{-5/7} \dot{m}_{17}^{-3/7} \tag{1}$$

where $\dot{m}_{17}$ is the accretion rate in units of $10^{17}\ \text{g s}^{-1}$, B_8 the surface dipole magnetic field in units of 10^8 G, M is the neutron star mass and R_6 is its radius in units of 10^6 cm. In estimating spin-up rates we shall neglect the torque on the magnetized neutron star from currents which flow through it as a result of the termination of its plasma-filled co-rotating magnetosphere at the Alfvén radius by conducting matter of different angular velocity. These may significantly change the spin-up and the dipole moment orientation but not equation (1). The accreted mass needed to achieve this limiting period is then

$$m_a \sim (0.22\,M_\odot) I_{45} (10^3 P)^{-4/3} (M/M_\odot)^{-2/3} \tag{2}$$

where I_{45} is the neutron star moment of inertia in units of $10^{45}\ \text{g cm}^2$, and the required accretion time is

$$T_a \sim 1.4\times(10^8\ \text{yr}) I_{45} (10^3 P)^{-4/3} (M/M_\odot)^{-2/3} \dot{m}_{17}^{-1} \tag{3}$$

The above equations are valid only if B is not so weak that the Alfvén radius shrinks to the stellar surface, that is, $B_8 \gtrsim 0.9\,(M/M_\odot)^{1/4} R_6^{-5/4} \dot{m}_{17}^{1/2}$; otherwise

$$P \sim 2.7\times10^{-4} \left(\frac{M}{M_\odot}\right)^{-1/2} \left(\frac{m_a}{M_\odot}\right)^{-1} R_6^{1/2} I_{45} \tag{4}$$

Immediately after accretion ceases the spun-up neutron star will have a spin-down rate $\dot{P}$ and a lifetime T_s of a freely rotating pulsar:

$$\frac{P^2}{T_s} \equiv P\dot{P} \sim \frac{8\pi^2 R^6 B^2}{3Ic^3} \tag{5}$$

This gives an initial

$$\dot{P} \sim (3.2\times10^{-20}) I_{45}^{-1} (10^3 P)^{4/3} (M/M_\odot)^{5/3} \dot{m}_{17} \tag{6}$$

and initial

$$T_s \sim (10^9\ \text{yr}) I_{45} (10^3 P)^{-1/3} (M/M_\odot)^{-5/3} \dot{m}_{17}^{-1} \tag{7}$$

During its radio pulsar phase the spun-up neutron star thereafter has increasing P and $\dot{P}^{-1}$ related by equation (5).

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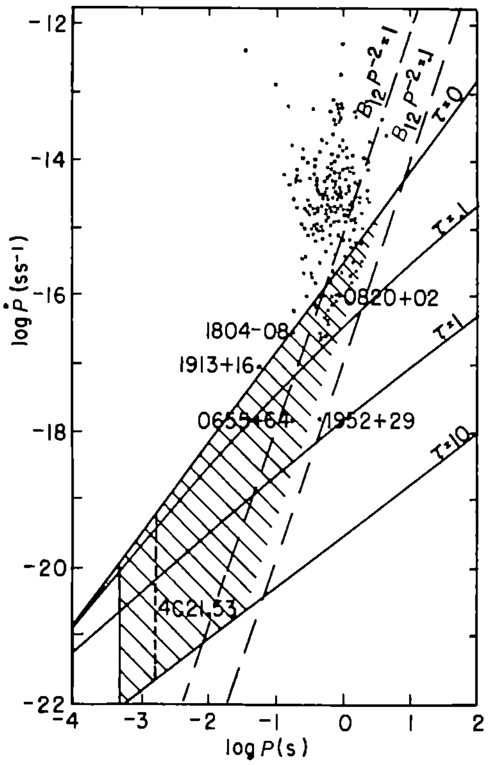

Fig. 1 The $\dot{P}$–P diagram for pulsars, after refs 1 and 17. Dots mark observed values with units as marked. Constant age curves for the new class of accretion spun-up pulsars are shown with $\dot{P}$ values given in units of $I_{45}^{-1}(M/M_{\odot})^{5/3}\dot{m}_{17}$ s s^{-1}. Their pulsar age τ is given in units of (10^9 yr) $I_{45}(M/M_{\odot})^{-5/3}\dot{m}_{17}^{-1}$. After birth at some point on the birth curve ($\tau = 0$) a pulsar is assumed to move on a $P\dot{P}$ = constant line; at age τ it is at the intersection of that line and the curve labelled τ. On hitting the appropriate $B_{12}P^{-2} = \eta$ line ($\dot{P}$ is in units of $R_6^6 I_{45}^{-1}$ s s^{-1} on these lines) it will die out as a pulsar. Thus, all pulsars of the new class should lie in a region similar to the shaded region of the diagram, bounded by the $\tau = 0$ line, the BP^{-2} line, the $\tau \sim 10$ (age of the Universe) line and the line of rotational break-up of neutron stars. These pulsars are confined to that region even when the pre-birth spin-up did not reach the $\tau = 0$ curve (that is, accretion stopped at P longer than equation (1)). It seems that many of the known neutron star binary X-ray pulsars would not become radio pulsars if accretion were to be interrupted now; and some will never become radio pulsars even with continuing spin-up if they are now rotating close to their equilibrium period of equation (1). The possible theoretical ($\dot{P}$, P) points for 4C21.53 lie on the dotted line.

Typical observed accretion rates onto neutron stars in binaries are $\dot{m}_{17} \sim 1$. Almost all radio pulsars[16,17] have $\dot{P}$ larger than initial values from equation (6) followed by spin down (unless $\dot{m}_{17}$ exceeds the Eddington limit by a typical three orders of magnitude). The new class of accretion spun-up pulsars would thus lie in a distinct region of the $P\dot{P}$ plane, bound by the initial conditions of equation (6) and corresponding roughly to observed $\dot{P} < 10^{-17}$ s s^{-1} (Fig. 1). We consider it very significant that this region contains all known binary pulsars as pointed out by Backus *et al.*[1] (Table 1), because binary radio pulsars with collapsed companions are the most promising candidates for old, low B neutron stars which have been spun up by accretion disks[18]. In Table 1 calculated initial $\dot{P}$ for $\dot{m}_{17} = 1$ are compared with observed $\dot{P}$ for these stars. Another potential candidate is the recently discovered radio pulsar 4C21.53 (ref. 19) for which a measured $\dot{P}$ is not yet available and for which there is no direct evidence supporting a present or past binary nature. We believe a history of past spin up by an accretion disk of a low B neutron star may be especially attractive because of the difficulty in understanding this star as a canonical young rapidly spinning pulsar. There appear to be problems in understanding an observation of a millisecond pulsar unless the neutron star had been relatively cold while it was being spun up and has also had a particularly low B ever since. Arguments for low B include the following: (1) A canonical B would give such a rapid spin down that the mere observation of such a pulsar would imply both an extraordinary birth rate and a very large pulsar population with periods between 10^{-3} and 10^{-1} s. We note also that no young supernova remnant is visible. (2) Such rapid spin down ($T_s \sim 1$–10^2 yr) would give internal heating from various mechanisms (crustal flow, superfluid vortex unpinning) that such a neutron star at Crab pulsar distances should have been readily observable by the Einstein Observatory. Observations, however, limit its surface temperature to $<1.5 \times 10^6$ K (D. Helfand and R. H. Becker, personal communication). (3) It would be difficult to account for the absence of observed energetic high energy emission. A spinning neutron star may be expected to have a total current flow and a maximum achievable potential drop along open field lines proportional to BP^{-2}. For the spun-up neutron star

$$\frac{B_{12}}{P^2} = (1.8\times10^2)(10^3 P)^{-5/6} R_6^{-3} (M/M_{\odot})^{5/6} \dot{m}_{17}^{1/2} \quad (8)$$

For $P \sim 10^{-3}$ s and Eddington limit accretion $\dot{m}_{17} \sim 10$, $B_{12}P^{-2} \sim 600$, essentially the same as that of the Vela pulsar and less if the spin-up accretion rate was less than Eddington. There is then no compelling reason to believe that the high energy emission supported by such currents should exceed those from Vela. A millisecond spun-up plusar which resembled Vela but was further away than the Crab pulsar would then certainly be presently undetectable in pulsed optical, X-ray and also in pulsed γ-ray emission below 30 MeV. In higher energy γ rays it may be observable because the period is determined. If $B_{12} \sim 1$, equation (8) would imply a value of $B_{12}P^{-2}$ almost 10^6 that of Vela. (4) It seems difficult for pulsar models which put the source of radiation within the magnetosphere to give GHz radiation when the velocity of light cylinder is near the stellar surface unless the surface B is small. Such models generally utilize e$^{\pm}$ pair production and/or relativistic ion flows. In either case a necessary condition is $B_{12}P^{-2} \gg n$, with n of order unity for e$^{\pm}$ pair production and 10^{-1}–10^{-2} for pure ion flow models. In either case we see from equation (8) that the inequality is easily satisfied for $P \sim 10^{-3}$ s. However, in most models, relevant plasma frequencies are sufficiently high throughout the magnetosphere that expected coherent radio emission is generally at frequencies much above 1 GHz.

If B is indeed to be anomalously low there is an additional reason that a cold, accretion, spin-up scenario is preferred to

Table 1 The binary pulsars and the superfast pulsar as accretion spun-up pulsars with $\dot{m}_{17} = 1$, $M = 1M_{\odot}$ and $I_{45} = 1$

Pulsar	P (ms)	$\dot{P}_{\text{theory}}$ (s s^{-1})	$\dot{P}_{\text{observed}}$ (s s^{-1})	B(G)	T_s (yr)	T_a (yr)
PSR 1913+16	59	7.3×10^{-18}	8.6×10^{-18}	2.1×10^{10}	2.6×10^{9}	6.0×10^{5}
PSR 0820+02	865	2.6×10^{-16}	1×10^{-16}	4.8×10^{11}	1.0×10^{8}	1.7×10^{4}
PSR 0655+64	196	3.6×10^{-17}	$<5\times10^{-18}$	8.6×10^{10}	1.7×10^{8}	1.2×10^{5}
4C21.53	1.5	5.5×10^{-20}	?	2.9×10^{8}	8.9×10^{8}	8×10^{7}

$\dot{P}_{\text{theory}}$ is calculated from equation (6) on assuming that the presently observed P is still the initial one. Because, in fact, both P and $\dot{P}^{-1}$ are expected to increase roughly by equation (5), one expects $\dot{P}_{\text{theory}} \gg \dot{P}_{\text{observed}}$.

a hot, supernova, one. Even if a high spin, low magnetic field pulsar could be formed in a supernova (and thus retain a high spin for times much longer than its cooling time), the large heat flux from that hot neutron star may still generate sufficiently large crustal magnetic fields by magnetothermal effects[10]. The neutron star would then spin down before the B field dissipated and thus have a period far longer than a millisecond. By contrast, in the presence of ohmic dissipation, the much smaller stellar heating during the accretion phase need not generate large magnetic fields[10].

We note that the predicted initial $\dot{P}$ of 4C21.53 as a superfast spun-up pulsar is $<5.5\times10^{-20}\,\mathrm{s\,s^{-1}}$ $(I_{45}^{-1}(M/M_{\odot})^{5/3}\dot{m}_{17})$. A larger $\dot{P}$ could indicate the presence of non-electromagnetic, for example, gravitational radiation torques.

The expected lifetime as X-ray sources of the low mass galactic bulge close binaries has been estimated to be of order 10^9 yr. If a large fraction do indeed end up as the single spun-up neutron stars of scenario (1) (many, perhaps, still in the dilute cloud formed by that part of the primary which was not accreted), the relative number of radio pulsars to neutron star X-ray sources would be of order $T_s/10^9\,\mathrm{yr}\sim1$. As there are of the order of 25 such neutron star X-ray sources[6] we might then expect a similar number of fast accretion-spun-up radio pulsars in the Galaxy. Almost all will be well beyond the $\sim6\times10^3$ light yr limit for pulsar detectability but to observe one or two does not seem implausible. Certainly it is among the galactic bulge X-ray sources that millisecond neutron star periods might be common.

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Persistent X-ray emission from a γ-ray burst source

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A quiescent X-ray source detected with the Einstein X-ray Observatory in a location consistent with that of an intense γ-ray burst[1–3] is shown here to be also consistent with the location of the 1928 optical transient[4], the likely optical counterpart of the γ-ray burst source GBS0117−29. The probable maximum X-ray luminosity is only $\sim10^{31}\,\mathrm{erg\,s^{-1}}$ and supports the hypothesis that either γ bursts are produced by accretion instabilities[5,6] or, if due to published models for a thermonuclear flash[7], they recur with intervals $\geqslant$50 yr. Such long recurrence times may be inconsistent with limits inferred from both the γ burst observations[8] and optical data[4].

The interplanetary burst network timing of γ bursts has yielded several relatively precise error boxes for source locations. One of these, the $\sim5\,\mathrm{arc\,min^2}$ location[1] of the γ burst detected on 19 November 1978 (GBS0117−29) led to the discovery of its probable optical counterpart from a brief optical transient found on a 1928 Harvard patrol plate[4]. The γ burst location was sufficiently precise that a search for an accompanying X-ray[1–3] and radio[9] counterpart could be carried out. A $\sim$9,000 s X-ray guest investigation observation[2,3] has been conducted with the IPC detector on the Einstein X-ray Observatory[10] on 1–2 July 1980. Preliminary analysis of these data yielded a 3.5σ source detection with a relatively large location uncertainty in which the optical transient was later found. These X-ray data have been recently reprocessed using an improved analysis system with the result that both the source detection and its positional coincidence with the transient optical counterpart are established. Further studies of this X-ray source, as well as upper limits from Einstein observations of several other γ burst positions, are reported elsewhere[11].

The new source location is plotted in Fig. 1, together with the position of the optical counterpart[4] and the original γ burst error box[1]. The X-ray source error box has a 90% confidence radius of 45 arc s and is centred 42 arc s north-west of the optical counterpart. The X-ray flux detected in the 'hard' 0.5–3.0 keV band is $1.0\times10^{-13}\,\mathrm{erg\,cm^{-2}\,s^{-1}}$. From the recent determination of log N–log S for high latitude X-ray sources[12], the probability that an X-ray source with flux $\sim1\times10^{-13}\,\mathrm{erg\,cm^{-2}\,s^{-1}}$ should be found within 1 arc min of the optical counterpart is only 1.7×10^{-3}. The consistency of the position of the persistent X-ray source with that of the optical transient link its identity to the larger source location region for the γ-ray burst. The 19 November 1978 burst was among the most intense ever recorded, which may account for the apparent absence[11] of other persistent X-ray counterparts (omitting the anomalous case of the 5 March 1979 burst apparently from the supernova remnant N49).

As the steady X-ray source was not detected in the 'soft' 0.15–0.5 keV band and no significant low energy absorption is expected at the −83° galactic latitude position it seems that the source is not a coronal emission source (for example, stellar X-ray emission from an M dwarf), but is instead an accretion source with a characteristically hard ($kT\geqslant2$ keV) spectrum. Too few counts were detected to allow useful spectral fits, hardness ratios, or low energy cutoffs to be determined from the data. A temporal analysis was carried out, however, and shows that the X-ray emission is consistent with a constant flux throughout the entire 9,000 s observation.

The probable optical counterpart of the 1928 optical transient and γ buster was detected[4] with quiescent magnitude $\geqslant$23 on the B and V 4-m plates of M. Liller. However, deeper searches carried out a month later with a 1.3-m telescope and CCD camera[13] gave upper limits of magnitude $\sim$25. Subsequent more sensitive CCD observations[14] have detected the quiescent and possibly variable emission from the source at magnitude $\sim$25. Thus the optical flux appears to be variable by a factor of at least 10 on time scales no more than $\sim$1 month. Presumably the quiescent X-ray flux is also variable although follow up X-ray observations are needed. The ratio of X-ray to optical luminosity is therefore poorly constrained but is probably in the range $L_x/L_{\mathrm{opt}}\simeq6$–60. This is similar to that found for low-mass X-ray binaries where the optical light arises from an accretion disk rather than the non-degenerate stellar com-

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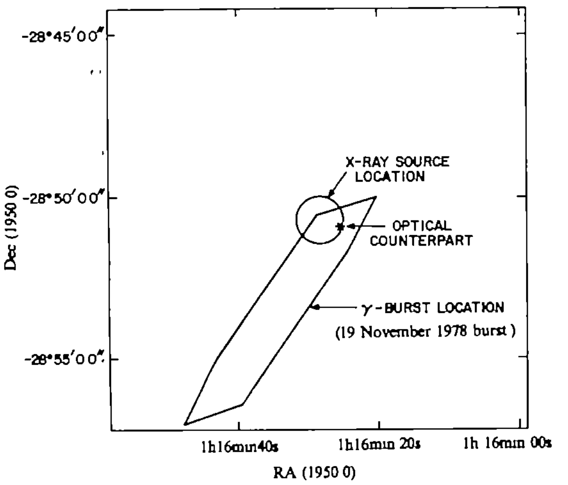

Fig. 1 Location (90% confidence error circle with 45 arc s radius centred at RA(1950) = 01 h 16 min 28.58 s, dec(1950) = −28°50′42″) of the quiescent X-ray source which is the probable counterpart for the γ-ray burster GBS0117−29. The interplanetary network position for the 19 November 1978 γ burst[1] and the location of the 1928 optical transient[4], the probable optical counterpart, are also shown.

panion. The L_x/L_{opt} value is probably not consistent with the value expected for an isolated neutron star accreting from an interstellar cloud but is intermediate between the typical values for compact binaries containing accreting degenerate dwarfs (with $L_x/L_{opt} \sim 1$) or accreting neutron stars (with $L_x/L_{opt} \sim 10^3$). Since the super-Eddington luminosities inferred for some γ bursts as well as the possible detection of both redshifted positron annihilation line radiation as well as possible cyclotron features all point strongly to a magnetized neutron star origin for γ bursts[15–17], we shall explore the consequences of this interpretation.

Our estimate for L_x/L_{opt} suggests that the system is underluminous (relative to compact binaries containing neutron stars) in X rays by perhaps a factor of 10. This could be due to the non-simultaneity of the X-ray and optical observations and/or to a highly variable accretion rate. Alternatively, it may indicate a relatively low accretion rate and a variable 'additional' optical contribution from cyclotron radiation in a $\sim 10^8$ G field at an effective Alfvén surface, where matter may accumulate before a burst. If the bursts are due to Rayleigh–Taylor like instabilities of $\sim 10^{19}$ g of matter (required for an $\sim 10^{39}$ erg total energy burst) accumulated in a magnetospheric shell, its radius would be $R \sim 2 \times 10^7$ for an assumed surface field $B \sim 1 \times 10^{12}$ G. The (assumed dipole) magnetic field in the shell would then in fact be $\sim 10^8$ G. Note that black-body radiation from the shell would not dominate the optical cyclotron radiation if the thermal temperature of the magnetospheric shell is $T \leq 4 \times 10^4 K$. Note also that such a 'shell' must, in fact, be a disk-geometry (a thick ring in the accretion disk?) since its large column density ($\sim 10^{28}$ cm^{-2}) would be opaque to quiescent X rays from the central neutron star.

The observed X-ray flux would require (for an assumed maximum distance of 1 kpc and $L_x \simeq 10^{31}$ erg s^{-1}) an accretion rate of $\simeq 1 \times 10^{-14}[d(1\text{ kpc})]^2 M_\odot$ yr^{-1}. This value is probably too low to be consistent with published nuclear flash models for γ bursts[7,16] unless the distance is substantially greater than ~1 kpc or the burst recurrence time is ≫50 yr, the accretion rate is highly variable, or further theoretical work on flash models shows that flashes yielding $\sim 10^{39}$ erg can be produced with a total accumulated mass of only $\sim 10^{20}$ g (as required for a ~5–10 yr recurrence time at the derived accretion rate). Such a large distance would make GBS0117−29 a halo object, whereas the isotropy and size distribution for γ bursts in general suggests[18] that they are an extended disk population in the Galaxy. Such a long recurrence time appears to be inconsistent with the detection of the optical burst[4]. A highly variable (factor of ≫50) accretion rate, while somewhat *ad hoc*, cannot be ruled out, and it might, in fact, be suggested by the variable optical flux. Once again, repeated X-ray observations are needed. However, if the average accretion rate and X-ray luminosity vary only by factors of ~3, as do most neutron star X-ray binaries (we may exclude X-ray transients, as their quiescent L_x/L_{opt} values are probably much lower than observed for this quiescent γ burster), we conclude that the accretion instability models[5,6] for γ bursts may be preferred.

If the quiescent X-ray emission from GBS0117−29 is typical, the summed X-ray flux from all such objects could be significant. For a currently extrapolated total of ~150 bursts yr^{-1} (ref. 19), and an assumed typical source distance of 300 pc and ~1 yr burst recurrence time, the volume density of γ bursters is $\sim 1.5 \times 10^{-6}$ pc^{-3}. This is within an order of magnitude of the value derived by Hertz and Grindlay[20] for low-luminosity ($\sim 10^{31}$ erg s^{-1}) accretion X-ray sources in the Galaxy as measured by the galactic plane survey carried out with the Einstein Observatory. Most of these low luminosity ($\sim 10^{31}$ erg s^{-1}) X-ray sources are probably cataclysmic variables but it is possible that some are the quiescent counterparts of γ burst sources. Given the similarities in space density, it is also possible that the γ bursters are a large fraction of the neutron star products of cataclysmic variable evolution wherein continued accretion onto a degenerate dwarf eventually violates the Chandrasekhar limit and collapse to a neutron star ensues. Such systems might also be expected to have evolved (by mass transfer) such low mass non-degenerate stellar companions that their absolute magnitudes would be consistent with the stringent limits ($M \geq 15$, assuming $d \leq 1$ kpc) recently derived[13,14] for the GBS0117−29 source.

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The tide in the seas of Titan

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The surface temperature of Titan[1] is about 95 K, between the melting point (90.6 K) and the boiling point (118 K) of methane at the total surface pressure of 1.6 bar. Voyager 1 IR observations[1,2] suggest a methane abundance in the lower atmosphere >0.19 of saturation. Comparison with the mean H_2O abundance in the Earth's atmosphere suggests[1] a CH_4 saturation level ≅0.5. Thus the volume mixing ratio of methane is probably about 6% and methane begins to condense out some 40 km above the surface. It is therefore a natural speculation that

methane exists in the liquid state on the surface of Titan. The atmospheric CH_4 corresponds to a liquid layer ~10 m deep. But it is unlikely that the buffer for atmospheric methane would have a capacity equal only to that of the atmosphere itself. On Earth, the quantity of water in the oceans is on average several times 10^4 the quantity of water vapour in the atmosphere. Thus, if Titan has oceans of liquid CH_4, they are probably >100 m deep. We argue here that, if this ocean exists, the present high eccentricity of Titan requires its depth to be >400 m, and that such an ocean can be detected by radar techniques.

The relief observed on other saturnian moons, such as Tethys and on jovian moons of comparable size, such as Ganymede, is ~1 km in scale[3]; we would anticipate comparable relief on the surface of Titan. Initially, therefore, we expect both land and sea on Titan. The interaction of UV light and charged particles with the titanian upper atmosphere produces a class of dark organic solids called tholins[4-7], which are expected over geological time to coat the land and accumulate as submarine deposits to a depth also in excess of 100 m (ref. 8).

Saturn will raise a significant tide in the seas of Titan, but because the satellite is probably in synchronous rotation with a period of 15.95 days the tide will be near-stationary and centred close to the Saturnward radius vector. If the solid body of Titan has not relaxed to hydrostatic equilibrium, then the maximum height of the solid body tide will be[9,10]

$$s = \frac{(5/2)(M_S/M_T)(R^4/a^3)}{1+\tilde{\mu}[1-(3\sigma/5\rho)]/[1-(\sigma/\rho)]} \qquad (1)$$

and the difference between the maximum heights of the oceanic and body tides will be

$$h = \frac{(M_S/M_T)(R^4/a^3)}{[1-(3\sigma/5\rho)]+[1-(\sigma/\rho)]/\tilde{\mu}} \qquad (2)$$

where M_S and M_T are the masses of Saturn and Titan, respectively; R is the surface radius of Titan; a is the semi-major axis of Titan's orbit; $\sigma = 0.45$ g cm^{-3} is the density of the putative titanian ocean; $\rho = 1.88 \pm 0.01$ g cm^{-3} is the mean density of Titan[3]; and $\tilde{\mu} = 19\mu/2\rho g R$, where μ is the rigidity of the solid body of Titan and g is the surface acceleration due to gravity. The greater the rigidity, the greater will be the amplitude h of the apparent oceanic tide.

The mean density of Titan is intermediate between the uncompressed densities of ice and rock; the solid body of the satellite is likely to consist of an icy mantle and a rocky core. The rigidity of water ice at temperatures near 100 K is 4×10^{10} dyn cm^{-2} (ref. 11). For methane and ammonia ices and their clathrates, μ should not be significantly less. The rigidity of rock in the interior of Titan will be larger. The central pressure is bounded by the following inequality[12], which is independent of the equation of state: $P_0 \leqslant P_c \leqslant (\rho_c/\rho)^{4/3}P_0$ where $P_0 = 3GM_T^2/8\pi R^4$. Allowing the central density, ρ_c, to range as high as 5 g cm^{-3}, we find $33 \leqslant P_c \leqslant 118$ kbar, a pressure range corresponding to that of the upper mantle of the Earth down to ≃350 km depth, a region[13] in which μ is $\sim 0.7\times 10^{12}$ dyn cm^{-2} and varies slowly with depth. Thus, we obtain a lower bound on μ and h by adopting the rigidity of water ice. Then, $\tilde{\mu} \gtrsim 6$, and

$$h \gtrsim 100 \text{ m} \qquad (3)$$

for the amplitude of the apparent, near-stationary, oceanic tide on Titan. Tides generated by the other saturnian moons have sub-millimetre amplitudes.

The possible existence of an ocean on Titan requires us to reconsider the effects of tidal dissipation. Previous studies[14] treat the effects of dissipation in the solid body of the satellite; but, as on Earth[13], solid body dissipation may be negligible compared with oceanic dissipation. There is no direct evidence that Titan is in synchronous rotation, but the time scale to achieve synchronicity is estimated[15] at 10^7–10^8 yr. The orbital eccentricity of Titan, $e = 0.0289$, is comparatively high. Tidal distortion decreases as the cube of the distance; thus, the amplitude of the tide raised by Saturn on the satellite will vary by a factor of $3e$, or 9% over its 15.95-day period. Titan rotates such that one face is directed towards the empty focus of its orbit, whereas the tide raised on it by Saturn is directed towards the planet. Consequently, the tide oscillates across the face of the satellite. The energy loss associated with these oscillations could, over the age of the Solar System, result in significant damping of the orbital eccentricity. As there appear to be no mechanisms available to increase e, the observed eccentricity can be used to establish limits on the surface topography and oceanic depths of Titan.

If the solid body of Titan has relaxed to near-hydrostatic equilibrium, then the maximum height of the mean solid body tide is given by equation (1) with $\tilde{\mu} = 0$. We have $s \leqslant 250$ m where the deviation from the upper limit is determined by the degree to which Titan is centrally condensed: the tide height is 250 m only if the density of the satellite is uniform[16]. It would also follow for hydrostatic equilibrium that $h = 0$, and if the orbit of Titan were circular then the depth of a shallow ocean would be everywhere the same and independent of the phase of the orbital period. However, as we have already noted, the orbit of Titan is far from circular. Since all deformations are small and can be added linearly, and since for short period oscillations the rigidity of the solid body cannot be ignored, then, regardless of the degree of relaxation of the solid body, the amplitude of the apparent oceanic tide will vary by an amount $3eh$ ($\gtrsim 9$ m) over a 15.95-day period, where h is given by equation (3).

Because the effect on e of the tide raised on Saturn by Titan is negligible[14], the time constant τ for the decay of the orbital eccentricity is given by[14,17]

$$\tau = \frac{2}{21}\frac{M_T}{M_S}\left(\frac{a}{R}\right)^5\frac{Q}{nk_2} \qquad (4)$$

where n, k_2 and Q are the mean motion, the Love number and the specific dissipation function of Titan respectively. Thus, $\tau = 4\times10^6\, Q/k_2$ yr and unless $Q/k_2 \gtrsim 1{,}200$, τ is less than the age of the Solar System and the present comparatively high value of e is difficult to understand.

The Love number is determined by the internal density and rigidity distributions and can be written[16] as $k_2 = (5/2)[H-(2/5)]$, where $H \leqslant 1$ and is unity only for a homogeneous satellite in hydrostatic equilibrium. Since $\tilde{\mu} \gtrsim 6$ the distortion of Titan's solid body is small and H can be estimated[16] from a point-core model as $H = (2/5)[1-(3\sigma/5\rho)]^{-1}$. Substituting, we find $k_2 = 0.17$. Hence, for $\tau > 4{,}600$ Myr, we must have

$$Q > 200 \qquad (5)$$

If the putative titanian ocean is global in extent, then the above lower bound on Q effectively rules out the existence of large land masses, which, as on Earth, would act as barriers to the tidal flow and would generate low values of Q (~10). If the ocean cover is nearly complete, then Q may be large (>200), but only if the mean depth D of the ocean is also large so that oceanic boundary layer turbulence is weak. Following Goldreich and Soter[18] we estimate that the rate of energy loss due to boundary layer turbulence is $\dot{E} = f\sigma\langle v^3\rangle$ erg cm^{-2}, where $f \approxeq 0.002$ is the coefficient of skin friction and v is the local velocity of the tidal current. The time-dependent part of the tide-raising potential of Saturn at some point (R, θ, ϕ) on Titan is

$$V = -\frac{GM}{a}\left(\frac{R}{a}\right)^2 3e$$
$$\times[\tfrac{1}{2}(3\sin^2\theta\cos^2\phi - 1)\cos nt + \sin^2\theta\sin 2\phi\sin nt] \qquad (6)$$

The first term in equation (6) excites the radial tide due to the variation in Saturn's distance and the second term is due to the optical libration of Titan. We estimate that the average tidal flow due to the radial tide alone is given by

$$\langle v^3\rangle^{1/3} = (2/105\pi^2)^{1/3}(3eh/D)nR \qquad (7)$$

where v^3 has been averaged over the surface of the satellite and over one tidal cycle. This estimate neglects the effects of coriolis forces, which act to increase the tidal flow rates; thus, our value of $\langle \dot{E} \rangle$ is an underestimate.

We set $\langle \dot{E} \rangle = ce^3$. Then, since the total orbital angular momentum of the system is conserved (the spin angular momentum of Titan is negligible), one can show that

$$\dot{e} = -ce^2/E \qquad (8)$$

where E is the total orbital energy. As the initial eccentricity of Titan's orbit must be <1, integration of equation (8) yields

$$|c| < |E|/et \qquad (9)$$

where t is the age of the system. If we now write $\langle \dot{E} \rangle = x\langle \dot{E}_r \rangle$, where $\langle \dot{E}_r \rangle$ is the energy loss due to the radial tide alone, then on substitution we find that equation (9) is satisfied only if

$$D > 300x^{1/3}\text{m} \qquad (10)$$

We estimate that, for the combined radial and libration tides, $x > 2$ and that the effects of coriolis forces will act to increase this limit further. Hence, we conclude, conservatively, that

$$D > 400\text{ m} \qquad (11)$$

Equations (1) and (2) for the tide heights assume that the kinetic energy of the tidal flow is negligible compared with the potential energy: the tides are assumed to be in static equilibrium. This is the case for the oceanic tide only if $D \gg (1/10)(nR)^2/g = 10$ m, a condition[19] which our previous arguments suggest is satisfied on Titan. If D were $\approxeq 10$ m, then the kinetic and potential energies would be comparable and we would expect enhanced dissipation and a reduced Q due to resonance in tidal basins. This is a further argument for a deep ocean. The velocity of the tidal current will be $v \simeq (h/4D)$ m s^{-1}, providing a possible source of erosion especially in shallows and on the equivalent of continental margins. It is therefore conceivable that the eccentricity-driven departure from a pure stationary tide has, over geological time, eroded away most of the land masses on Titan, and is thereby responsible for the large value of Q [equation (5)].

Accordingly, there seem to be two internally consistent possibilities: (1) a near-global methane ocean >400 m deep that has inundated or eroded away most of the land on Titan, and (2) almost no methane ocean at all. In the latter case, the position of the surface of Titan near the liquidus in the CH_4 phase diagram will have to be considered a distracting coincidence, and the observed atmospheric methane must maintain itself against photolysis by some mechanism other than the equilibrium vapour pressure resupply from a methane ocean[8]. Fortunately, the choice between these alternatives can be made by remote observations.

The dielectric constant, ε, at microwave frequencies of liquid methane at the surface temperature of Titan is[20] about 1.7. As the square of the imaginary part of the complex refractive index, $k^2 \ll (\sqrt{\varepsilon} - 1)^2$, the normal-incidence Fresnel equation yields a microwave reflectivity for a smooth methane ocean of about 0.017. For many organic solids $\varepsilon \approxeq 2.6$ at visible wavelengths, increasing to 4–6 at microwave frequencies. X- and S-band values of ε for tholins have not yet been measured, but are likely to be in this range. The corresponding normal-incidence Fresnel reflectivities, with the same condition on k, range from 0.05 to 0.18. Thus we expect a contrast between the microwave reflectivity of methane oceans and tholin-covered land ranging between a factor of several and a factor of 10. If the land is not entirely inundated and the solid body has not relaxed to near-hydrostatic equilibrium, then we might expect a systematically higher radar reflectivity near the 0° and 180° meridians. If the solid body has relaxed then we might expect small variations in the disk-integrated reflectivity at a constant central meridian longitude with a period of 15.95 days.

Preliminary attempts to observe Titan near elongation with the 305-m Arecibo telescope during the 1979 opposition set only an upper limit of unity to the radar reflectivity (D. Campbell and S. Ostro, personal communication). Considerably higher precision can be expected during the next Arecibo apparition of Titan in the mid-1990s, which can also check whether Titan is in fact in synchronous rotation. These considerations make still more interesting recent proposals to examine Titan, from orbit or from a probe-carrying flyby bus, with a spaceborne radar reflectometer.

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Design of a 13% efficient n-$GaAs_{1-x}P_x$ semiconductor–liquid junction solar cell

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We report here the design of the most efficient non-aqueous semiconductor–liquid junction solar cell studied to date. Our approach involves the use of ternary semiconductor electrodes made from solid solutions of a large band gap material, GaP, and a small band gap material, GaAs. We demonstrate here that photoanodes consisting of such materials are capable of simultaneously yielding high open circuit voltages and favourable wavelength response to the solar spectrum. A few n-type semiconductor–liquid junction solar cells in aqueous solutions have been reported to yield high (>10%) solar-to-electrical conversion efficiencies[1–3]. However, for most materials, rapid photoanodic corrosion dominates the interfacial photochemistry[4–8]. Non-aqueous solvent systems can suppress electrode decay due to corrosion[4,7,8]; but modest (<6%) conversion efficiencies have been observed for all photoanodes studied in solar irradiation conditions[9–13]. The photoanodes used here yield over 13% solar-to-electrical conversion efficiencies, or more than double the efficiency of any other non-aqueous semiconductor–liquid junction solar cell previously reported.

We have studied a set of compositions of $GaAs_{1-x}P_x$, $0 < x \leqslant 1$, obtained as epilayers deposited on n^+-GaAs or n^+-GaP substrates by vapour phase epitaxy techniques. The epilayer is thick enough (0.1 mm) to prevent any photoactivity in the substrate. This has been verified by the lack of photocurrent on excitation of $GaAs_{1-x}P_x$ ($x \geqslant 0.3$) samples on n^+-GaAs substrate with light $\lambda \geqslant 750$ nm.

Composition of the epitaxial layers used was verified by electron microprobe analysis and found to be: $GaAs_{0.85}P_{0.15}$, $GaAs_{0.72}P_{0.28}$, $GaAs_{0.61}P_{0.39}$, $GaAs_{0.38}P_{0.62}$, $GaAs_{0.31}P_{0.69}$, and

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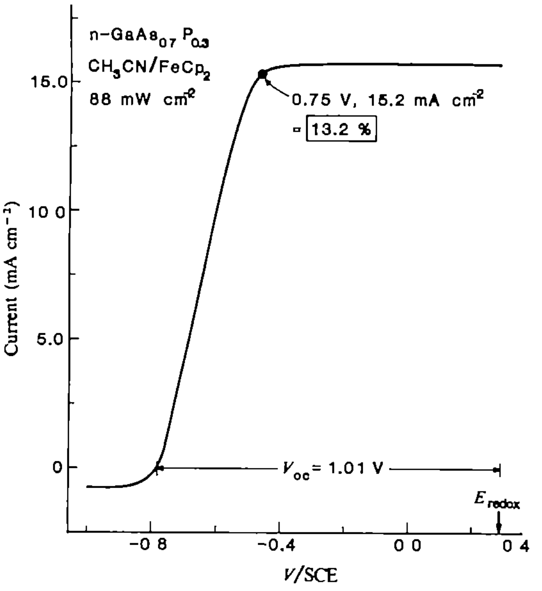

Fig. 1 Current–voltage curve at 50 mV s^{-1} (88 mW cm^{-2}) of a sample of n-GaAs$_{0.72}$P$_{0.28}$ in 0.1 M Fc/0.5 mM Fc$^+$/ACN. The electrolyte is 1.0 M (Et$_4$N) BF$_4$, and the solution is stirred magnetically. The solution potential is +0.22 V/SCE, and photocurrent negative of this potential represents net conversion of light into electricity.

GaAs$_{0.05}$P$_{0.95}$. Increasing the P content of the samples increases the band gap, E_g, in a monotonic, but not linear fashion, from 1.44 eV ($x = 0$) to 2.25 eV ($x = 1$) (ref. 14). A direct-to-indirect gap transition occurs at composition $x = 0.44$, with n-GaP having an indirect gap of 2.25 eV and a direct gap of 2.75 eV. Samples (0.1 cm^2) were mounted as photoelectrodes by conventional techniques. Ohmic contacts were formed by evaporation of In at 1×10^{-6} torr and annealing under N_2 at 400 °C for 15 minutes. Potentials were recorded versus either a standard calomel reference electrode or a Pt wire placed ~1 cm from the working electrode. We have made no effort to minimize or instrumentally correct for the uncompensated ohmic resistance (~50 Ω) in this cell design; thus the efficiencies reported here are conservative by 10–20%. Light sources were either the Sun, a calibrated W-halogen ELH-type lamp with a ground glass diffuser, or a 5 mW He/Ne laser. Light intensities were measured with a Solarex secondary standard silicon cell and verified to within 10% by an Eppley thermopile. For samples with $E_9 < 2.0$ eV, we consistently find differences in efficiency measurements between our calibrated ELH lamp and actual sunlight of <10%. The samples were etched with 1 : 1 HF/H_2O_2 for 15 s, rinsed with H_2O, and air dried before use.

W-halogen irradiation (88 mW cm^{-2}) of a sample of n-GaAs$_{0.72}$P$_{0.28}$ (not deliberately doped; $N_D = 3.3\times10^{15}$) in the solution 0.1 M ferrocene (Fc), 0.5 mM Fc$^+$ in dry acetonitrile (ACN) solvent (electrolyte 1.0 M $(C_2H_5)_4N^+BF_4^-$) typically yields the current–voltage profile represented in Fig. 1. We observe an open-circuit voltage, (V_{oc}), of 1.01 V, a short-circuit current of 15.7 mA cm^{-2}, and a fill factor of 0.73, leading to an optical-to-electrical conversion efficiency of 13.2% (15.2 mA cm^{-2} and 0.76 V at the point of maximum power). In natural sunlight (64 mW cm^{-2}), we observe similar behaviour, and conversion efficiencies of 12.5–13.0% are typical.

For comparison with other literature data, we have also recorded the current–voltage characteristics at 632.8 nm with a He–Ne laser. Values are consistent with the W lamp data, and at intensities of 20 mW cm^{-2} we observe over 22% efficiency for conversion of monochromatic 632.8 nm light to electricity.

The extremes of our series, n-GaAs and n-GaP, have been previously studied in ACN electrolytes. n-GaAs, with E_g = 1.44 eV, is ideally suited for terrestrial solar applications; however, in similar non-aqueous cells, it is reported to yield modest operating voltages (0.25 V) (ref. 15), low quantum efficiencies (0.3) (ref. 16), and fill factors which decline at irradiation levels above 0.01 suns[15], leading to solar conversion efficiencies of 2.4% (ref. 15). n-GaP is reported to yield output voltages of up to 0.8–1.0 V (ref. 17). However, its E_g (indirect) of 2.25 eV leads to low short-circuit currents and poor solar efficiency. The goal of our studies with GaAs$_{1-x}$P$_x$ was to maintain the high output voltage characteristics of n-GaP with the favourable solar wavelength response of GaAs.

We observe much larger values of V_{oc} with n-GaAs$_{0.72}$P$_{0.28}$ than reported for n-GaAs. A comparison of our GaAs$_{1-x}$P$_x$ data with the performance of GaAs (refs 15, 16) suggests that substitution of P for As in the GaAs lattice eliminates most of the sources of inefficiency in ACN solvent. Consistently, we observe high values of the fill factor, >0.70 for all samples with $x \geq 0.25$, which are fairly independent of light intensity even at irradiance levels near 1 Sun.

A plot of V_{oc} at 88 mW cm^{-2} in Fc/Fc$^+$/ACN solution as a function of x (Fig. 2) shows that even for low levels of P, ($x = 0.3$), V_{oc} has increased to 1.01 V. Further increases in x lead to smaller changes in V_{oc}. As expected, the short-circuit currents measured with our W-halogen irradiation source decrease with increasing value of E_g (ref. 18). Thus, there is a maximum P content for optimum cell efficiencies, and for the range of samples studied, this occurs with GaAs$_{0.72}$P$_{0.28}$, E_g = 1.77 eV. An E_g of 1.4 eV is optimum for AM1 solar conversion devices[19], and increasing the E_g to 1.77 eV decreases the maximum theoretical AM1 conversion efficiency from 36 to 32% (ref. 19). However, for these GaAs$_{0.72}$P$_{0.28}$ samples, the increased values of both V_{oc} and the voltage at maximum power (V_{pmax}) compared with those reported for n-GaAs in non-aqueous systems results in much higher efficiencies for the larger E_g materials used here (n-GaAs$_{0.72}$P$_{0.28}$: $V_{oc} = 1.01$, $V_{pmax} = 0.76$; n-GaAs: $V_{oc} = 0.62$, $V_{pmax} = 0.25$).

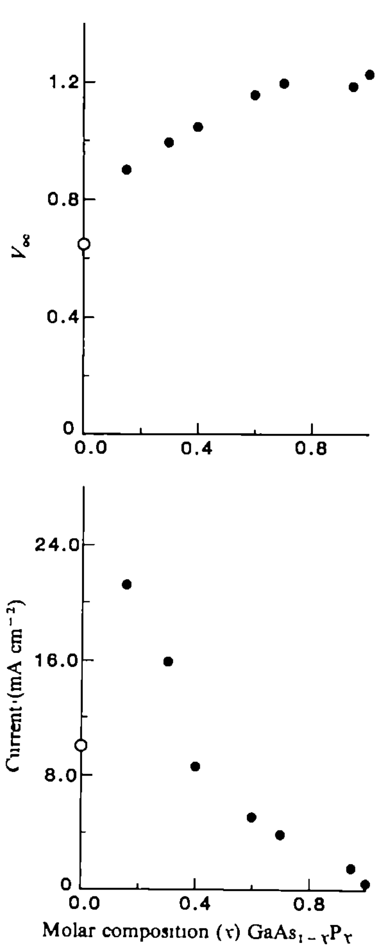

Fig. 2 Plot of open-circuit voltage and short-circuit current against composition for n-GaAs$_{1-x}$P$_x$. All measurements were performed in conditions identical to those in Fig. 1. ○, Previously reported data for n-GaAs, taken from refs 15 and 16.

We have studied changes in V_{oc} for our n-$GaAs_{0.72}P_{0.28}$ as a function of solution potential, $E°$, with a variety of redox reagents. As measured by either cyclic voltammetry or steady-state current–voltage curves, we find that V_{oc} is relatively constant (0.95 ± 0.10 V) for a range of redox couples whose $E°'$ spans more than 1.5 V. For example, we find that the photoanodic peak in a cyclic voltammogram (a measure of V_{oc})[20] for n-$GaAs_{0.72}P_{0.28}$ appears at -0.71 V/SCE for Fc oxidation (E_p at Pt $= +0.30$ V/SCE) and for the leuco form of methyl viologen, MV°, at -1.86 V/SCE (E_p at Pt $= -0.85$ V/SCE). Such behaviour has been observed for several semiconductor–liquid interfaces, and has been attributed to either carrier inversion at the surface[21] or to a high density of surface states[22] which act to pin the surface Fermi level to values independent of the solution $E°$. Such situations, as in non-aqueous n-GaAs systems,[15] limit the maximum V_{oc} attainable and can yield lower than optimum conversion efficiencies. Substitution of P for As in the lattice of n-GaAs leads to stronger bonding in both the bulk and surface regions of the material. Initial increases in x thus produce a highly favourable shift in factors controlling V_{oc}, with only a slight increase in E_g. Further increases in x yield little improvement in V_{oc}, only serve to increase the value of E_g, and thereby lower the overall efficiency.

Deliberately dried ACN solutions (3-Å sieves) yield extremely reproducible (±5%) current–voltage parameters over periods of hours with no visible surface damage or deterioration of cell properties. As reported for n-GaAs in ACN, injection of small amounts of H_2O (for example 75 mM H_2O) leads to rapid photocurrent decay, presumably due to oxide formation[15]. At present, we have observed stable photocurrents (±10%) at 15 mA cm^{-2} with n-$GaAs_{0.72}P_{0.28}$ for passage of over 10^3C cm^{-2} at short circuit with no apparent weight loss of the crystal or visible surface damage. We have ruled out substantial photocorrosion with experiments using thin (5 μm) epilayers of n-$GaAs_{0.72}P_{0.28}$ on n^+-GaAs. Assuming a six electron decomposition process, consumption of the entire epilayer in this 2.5×2.5 mm crystal would require ~1 C of charge. After passage of over 30 C through this particular crystal, we still observe the V_{oc} and wavelength response of the n-$GaAs_{0.72}P_{0.28}$ epilayer with no apparent contribution from the substrate. Photocorrosion processes would thus appear to be minimal. Long-term experiments are in progress, and the ultimate stability of these systems will probably depend on the extent to which water can be excluded from the electrolyte, solvent, and cell environment.

We note that our 13% efficiencies have been obtained on electrode surfaces with no deliberate attempts to optimize fully donor densities, reflectivity losses, and surface treatments. Such procedures have been shown to improve the performance of several aqueous photoelectrochemical systems in conditions similar to those utilized here[23]. We are developing such optimization procedures for our photoelectrodes, as well as studying the effect of ion implantation of P into the surface region of n-GaAs. The high efficiencies of the $GaAs_{1-x}P_x$ system demonstrate that properly designed non-aqueous electrolyte based photoelectrochemical cells can provide efficiencies competitive with aqueous systems. Moreover, control of V_{oc} by varying bulk composition can be an important approach to the design of efficient semiconductor–liquid junction cells, as evidenced by the dramatic improvements in efficiency observed with our photoelectrode materials. At present, the 13% solar conversion efficiency for n-$GaAs_{0.72}P_{0.28}$ system represents the highest value reported for any semiconductor–liquid junction cell in actual solar conditions.

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^{36}Cl bomb pulse measured in a shallow ice core from Dye 3, Greenland

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Nuclear weapons tests at oceanic sites during the 1950s produced a large amount of ^{36}Cl ($t_{1/2} = 3.0 \times 10^5$ yr) through neutron capture of ^{35}Cl in seawater. Part of this anthropogenic ^{36}Cl was injected into the stratosphere from where it was redistributed throughout the Earth. This pulse of ^{36}Cl was first detected in rainfall by Schaeffer *et al.*[1]. The global deposition rates are several orders of magnitude larger than the natural pre-and post-bomb ^{36}Cl production rate that reflects cosmic-ray spallation of atmospheric ^{40}Ar. Because ^{36}Cl is not a fission product, the anthropogenic ^{36}Cl is produced mainly in marine tests carried out on small islands and barges where a large amount of seawater chlorine is present to serve as a target. This target element requirement leads to a temporal dependence different from the other bomb produced isotopes[2–4]. We have determined the temporal dependence of ^{36}Cl fallout by measuring the depth profile of ^{36}Cl in an ice core from Dye 3 Greenland (65° 11′ N, 43° 50′ W) using tandem accelerator mass spectrometry[5]. We show here that the results agree well with a calculation of the ^{36}Cl produced and injected into the stratosphere by the tests.

The samples were prepared from a 100-m ice core drilled at Dye 3 in 1980 as a side project of the US–Danish–Swiss Greenland Ice Sheet Program (GISP). Based on space charge measurements[6], the core was cut at the site into pieces containing the precipitation of approximately 1 yr. The $\delta^{18}O$ profile determined later (W. Dansgaard, personal communication) showed that the actual intervals ranged from 0.5 to 1.5 yr (Table 1). The sample weights ranged from 0.9 to 2.2 kg. Before adding Cl carrier (1.52 mg Cl) and Be as carrier (for ^{10}Be measurements that will be reported elsewhere) to the melted ice, an aliquot was taken for chloride analysis. The natural chloride content, measured by ion chromatography for several of the samples, was found to be about 50 μg per kg-ice and thus contributed only ~3% to the chloride content of the prepared sample. The chloride was separated by precipitation of AgCl and purified as described elsewhere[7].

The ^{36}Cl concentrations were measured using the University of Rochester's MP tandem van de Graaf accelerator facility. A major improvement to the system since our previous ^{36}Cl

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Table 1 ^{36}Cl content of Dye 3 ice samples

Sample	Weight (kg)	Interval (yr)	Accumulation* (m)	Season–year†	^{36}Cl/Cl ($\times10^{-15}$)	Atoms per kg-ice ($\times10^6$)
B1	1.50	1.25	1.23	A76–W77/78	250±100	4.3±2
B2	1.43	0.75	0.90	W75/76–A76	210±100	4.0±2
B3	1.17	0.50	1.16	S75–W75/76	680±100	15.0±3
B4	1.13	0.86	1.29	S/A74–S75	590±100	14.0±3
B5	1.29	0.88	1.30	A73–S/A74	280±100	6.0±3
B6	1.22	0.36	0.90	Sp/S73–A73	310±100	6.7±2
B7	1.25	0.88	1.50	S72–Sp/S73	880±100	19.0±3
B8	1.47	1.00	0.96	S71–S72	530±100	9.6±2
B9	1.45	1.00	0.59	S70–S71	1,480±150	27.0±3
B10	0.90	0.50	1.37	W69/70–S70	610±100	18.0±3
B12	1.09	0.75	0.89	Sp68–W68/69	1,430±300	34.0±7
B14	0.98	0.63	0.88	Sp/S66–W66/67	2,330±350	62.0±9
B16	2.20	1.13	1.18	W63/64–W/Sp65	8,130±1,800	96.0±22
B18	1.27	1.25	0.54	W61/62–S63	7,020±700	150.0±15
B19	1.73	1.00	1.05	W60/61–W61/62	10,800±1,000	165.0±17
B20	2.01	1.25	0.94	A59–W60/61	20,900±1,500	274.0±20
B22	1.65	1.00	0.90	A57–F58	14,700±3,000	230.0±50
B23	1.30	0.75	0.96	W56/57–A57	13,400±600	272.0±0.15
B24	1.33	1.00	0.61	A/W55–W56/57	16,700±900	330.0±20
B25	1.59	0.86	1.01	W54/55–A/W55	16,100±1,600	267.0±30
B26	1.90	1.00	0.90	W53/54–W54/55	4,400±650	62.0±9
B27	1.43	1.25	0.50	A52–W53/54	1,300±100	24.0±2
B28	1.63	1.25	0.88	S51–A52	470±100	7.5±2
B29	1.21	0.81	0.59	S/A50–S51	250±100	5.4±2

* Actual accumulation depth of ice.

† A, Autumn; W, winter; Sp, spring; S, summer.

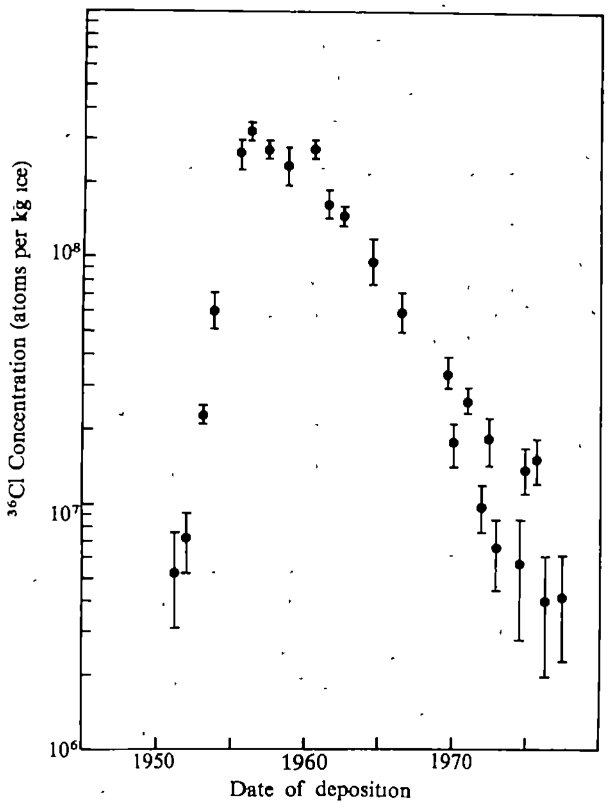

Fig. 1 The ^{36}Cl profile in an ice core drilled at Dye 3, Greenland, showing the large pulse that resulted from activation of ^{35}Cl during marine nuclear weapons tests. The age scale was determined by counting the seasonal variations in δ ^{18}O.

measurements in polar ice[7,8] is a new Cs sputter ion source[9] designed specifically for tandem accelerator mass spectrometry. This source produces a high negative ion current (10–20 μA) with improved stability. For this project the quantity of carrier was kept small (final weights ranged from 0.5 to 1 mg Cl) to ensure that the ^{36}Cl/Cl ratios before and after the bomb pulse would be well above background levels. Even with the new ion source these small samples caused some difficulties with beam stability. This is reflected in the quoted 1σ uncertainties (Table 1) which range from 5 to 50% and are generally larger than statistical errors. The background level for this run was fairly high and variable because of contamination during the sample preparation or loading and/or cross contamination during the measurements. The addition of more carrier to reduce the ^{36}Cl/Cl for the samples at the deposition peak would have alleviated these problems. From the experience gained in this run and others[7] we feel that 3–5% errors are possible with 2–3 mg Cl samples and that 1-kg ice samples should be adequate for measuring pre-bomb ^{36}Cl in polar ice cores.

The results given in Table 1 and Fig. 1 are expressed as atoms of ^{36}Cl per kg of ice and have not been corrected for the variable precipitation rate (Table 1). The ^{36}Cl/Cl ratios were reduced by the background value, which ranged from 70 to 200×10^{-15}, and were normalized to an NBS standard. The results show that ^{36}Cl concentrations at Dye 3 increased by a factor of more than 100 from pre-bomb levels. From 5×10^6 atoms per kg-ice in 1950, concentrations increased rapidly to 300×10^6 atoms per kg-ice in 1955 and then held that level until about 1960. Between 1960 and 1970 the ^{36}Cl decreased exponentially with a mean life of about 3 yr. Because of the high background and the lack of data points before 1950 we can only give an upper limit of 5×10^6 atoms kg^{-1} for the pre-bomb production rate for cosmogenic ^{36}Cl.

The integral of the ^{36}Cl bomb pulse (Fig. 1), when multipled by an average accumulation rate for Dye 3 (ref. 10) of 50 g cm^{-2} yr^{-1}, gives a total fallout at Dye 3 of 1.3×10^8 atoms cm^{-2}. This will now be compared with an estimate of the ^{36}Cl produced in oceanic tests and transported to Greenland. Table 2 lists the marine tests with a yield over 1 Mton that have been published[11–13], although we have not included the seven bombs >1 Mton detonated by the UK at Christmas Island (2° N, 127° W) during the Grapple series of tests because these were small air bombs whose contribution of ^{36}Cl to the strato-

Table 2 ^{36}Cl production in marine nuclear weapons tests

Test	Date	Explosive yield (Mton)	Location*	Predicted total ^{36}Cl production (kg)
Mike	31 October 1952	10.4	E	19.9
King	15 November 1952	>0.2	E	0.4
Bravo	28 February 1954	15	B	28.7
Zuni	27 May 1956	3.53	B	6.8
Tewa	20 July 1956	5.0	B	9.6
Koa	12 May 1958	1.37	E	2.6
Oak	26 June 1958	8.9	E	17.0
Castor	15 January 1968	0.5	T	
Canopus	24 August 1968	2.5	T	
Procyon	8 September 1968	1.0	T	
Dragon	30 May 1970	0.1–1	T	
Licorne	3 July 1970	~1	T	
Rhea	14 August 1971	~1	T	

* E, Pacific Proving Ground, Enivetok Island (11° N, 162° E); B, Pacific Proving Ground, Bikini Island (11° N, 165° E); T, Tuamotu Archipelago (21° S, 137° W).

sphere was probably quite low. Also, the large USSR tests at Novaya Zemlya Island (75° N) during 1960–61 were not included. It is not known if these test sites were near the ocean, or whether they were air or surface tests. They do not appear to have contributed in a significant way to the total ^{36}Cl. The contribution of ^{36}Cl from surface tests would not be significant since the Cl content of soil and rock is two orders of magnitude less than that for seawater.

Assuming that 2×10^{26} neutrons are produced per megaton[14], that half of the bomb neutrons enter the water, and that 32% of the neutrons that enter the water produce ^{36}Cl (ref. 15), we obtain the total ^{36}Cl produced from each premoratorium test (Table 2, column 5). For the simplest calculation, we can assume that all of this ^{36}Cl (85 kg) enters the stratosphere and is deposited uniformly over the Earth. This results in a global average deposition of 2.8×10^{8} atoms cm^{-2}. This calculated accumulation is about a factor of two higher than our measured value at Dye 3. It is, however, well documented that the distribution of fallout is not globally uniform. Measurements of ^{90}Sr deposition between 1958 and 1966 show that the deposition rate between 60° N and 70° N is actually 1.3 times lower than the global average[16]. Correction for this nonuniformity reduces the discrepancy between measured and calculated ^{36}Cl deposition to less than a factor of two. We consider this to be excellent agreement since: (1) some of the ^{36}Cl produced may not enter the cloud; (2) ^{90}Sr may have a different particle size distribution from ^{36}Cl, (3) a site dependence of fallout within Greenland of a factor of two has been observed[3], and (4) the effect of precipitation rate has been ignored.

Our Dye 3 result can also be compared with measurements of ^{36}Cl in rain and groundwater from the mid-latitudes. The ^{36}Cl deposition rate measured[1] in Long Island, New York rain collected during the bomb peak (1957–60), when corrected[17] for the precipitation rate, is 2.3 atoms cm^{-2} s^{-1}. A recent measurement of ^{36}Cl in groundwater from Ontario, Canada[17] gave a peak value of 4.8 atoms cm^{-2} s^{-1}. Our result for Dye 3, 0.5 atoms cm^{-2} s^{-1}, is a factor of 5–10 lower than these mid-latitude values. This is probably due to a combination of the latitude dependence of the stratospheric fallout and the additional contribution from direct tropospheric fallout from the Northern Hemisphere tests at lower latitudes.

The predicted mean lifetime of ^{90}Sr in the stratosphere using the model of Peterson, which takes into account the altitude and latitude of injection, is about 2 yr. This value is somewhat less than our measured value of ~3 yr for ^{36}Cl. Although this discrepancy may not be significant (an exponential with a slope of 2 yr is almost within the error bars) possible explanations are (1) contributions from additional tests during the period 1960–70 of which we are unaware and (2) fractionation between Cl and Sr in the transport out of the stratosphere.

After the Limited Test Ban Treaty in 1963, there were several smaller atmospheric tests listed in Table 2. Because these tests were smaller, higher above the ocean, and in the Southern Hemisphere their contribution to the total anthropogenic ^{36}Cl is small and hard to estimate accurately. However, they may be the cause of the small peaks seen in the data in the early 1970s.

It may be possible to use variations in the production of cosmogenic ^{36}Cl to study past solar activity as seen through the Sun's modulation of the galactic cosmic ray flux. The atmospheric residence time for ^{36}Cl is much less than that for ^{14}C so that time resolution should be better and amplitude variations should be larger. Our upper limit for pre-bomb ^{36}Cl fallout is 8×10^{-3} atoms cm^{-2} s^{-1} which is consistent with predictions[18,19] of $1\text{–}3\times10^{-3}$ atoms cm^{-2} s^{-1}. Further measurements with older ice are planned.

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Thermonuclear ^{36}Cl pulse in natural water

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Radionuclides produced by atmospheric testing of nuclear weapons have been used as environmental tracers for the past 30 years. ^{185}W and ^{90}Sr have proved valuable in monitoring atmospheric circulation[1], ^{14}C has been applied to oceanic mixing[2], and ^{3}H has been routinely used as a tracer in the hydrologic system. Tandem accelerator mass spectrometry[4] has made possible measurements of several long-lived radionuclides that were previously virtually undetectable. One of the potentially most valuable of these for studying the movement and mixing of groundwater and surface water is ^{36}Cl. ^{36}Cl is produced continuously in the terrestrial environment in very small amounts by spallation of argon by cosmic radiation and by the capture of naturally produced neutrons by ^{35}Cl. It was also produced in much larger amounts by neutron activation of seawater and released into the environment during atmospheric

thermonuclear tests. This ^{36}Cl pulse has many potential applications as a tracer in natural water systems. Here, numerical modelling and analyses of water samples indicate that in the mid-latitudes the fallout peak was 3 orders of magnitude above the natural background, and that the period of enhanced ^{36}Cl fallout was 1953 to about 1964.

Chloride is widely used as a natural and artificial tracer in studies of freshwater systems. In most conditions, it is negligibly sorbed on solid particles, which greatly enhances its potential value. However, the timing and size of the ^{36}Cl fallout pulse, which have not been reported previously, have to be known to make best use of the bomb ^{36}Cl as a tracer.

^{36}Cl differs from many other bomb-produced tracers in that it is neither a product of direct fission nor of fusion. Virtually all bomb ^{36}Cl originates from thermal neutron activation of ^{35}Cl. Sufficient chloride to produce measurable global ^{36}Cl fallout is present only when nuclear explosions are detonated near seawater. Thus only reported explosions on barges or small islands, of sufficient yield to penetrate the stratosphere, have been used as input to our model. Explosion data were taken from refs 5, 6. Neutron activation production in seawater has been calculated by Dyrssen and Nyman[7]. Cloud heights for some tests are published by Glasstone[8]; if not available, they were estimated according to Peterson[9].

The fallout from these stratospheric injections was simulated by means of the HASL atmospheric box model of Krey and Krajewski[10], modified to simulate global, rather than hemispheric, fallout. The model distinguishes between the stratosphere above 21 km, the stratosphere below 21 km, and the troposphere. The fallout from one to the next is considered to be a first-order rate process, with rate constants derived from ^{109}Cd and ^{90}Sr fallout data[10].

Table 1 ^{36}Cl fallout rates calculated from ^{36}Cl concentrations in modern water

Location	Date collected	Precipitation evapotranspiration (mm)	Measured concentration (atoms ^{36}Cl l^{-1}) ($\times 10^{-7}$)	Calculated fallout (atoms ^{36}Cl m^{-2} s^{-1})
Pre-1953 water				
(Before nuclear testing)				
Arizona[4]		300/98%		
Tucson City Well B8	January 1979		11.7	23
Tucson City Well C13	January 1979		9.6	18
Tucson City Well A31	January 1979		6.6	12
Monkey Springs	July 1979		8.1	15
Benson Well	July 1979		7.4	14
St David Well	July 1979		6.5	12
		Mean	8.4	16
Madrid Basin Wells		400/98%		
535-7a			7.1	18
535-7b			8.3	22
535-5a			5.6	15
		Mean	7.0	18
Texas Well		660/98%		
AL-68-59-504	December 1979		2.9	12
Atmospheric nuclear testing period (1953–64)				
New York (Long Island)[11]		1150/0%		
Rainfall	August 1957		77	28,000
	September 1957		183	67,500
	April 1959		24	8,900
	May 1959		1.02	370
	February 1960		26.3	9,600
		Mean	62	23,000
Shallow Well	August 1957		64	23,500
Post-1964 water				
Arizona[4]		300/98%		
San Pedro River	July 1979		9	17
Arizona (Tucson)		300/0%		
Rainfall	February 1982		0.26	25
Rainfall	March 1982		0.31	29
California (La Jolla)[19]		250/0%		
Rainfall	March 1979		0.2	16
Mixed samples				
Arizona		300/98%		
Campbell Ave Well	January 1979		158	310
Texas Well		660/98%		
AL-61-58-803	December 1979		20	85
New York				
Lake Ronkonkoma[11]	September 1960	1,150/50%	26.8	4,600
Lake Ontario[4]	May 1978	750/50%	9.5	1,100
	June 1978	750/50%	5.4	640
Vermont[11]		1,150/50%		
Stream	September 1957		26.8	4,600
Nevada[20]		380/98%		
Truckee River	August 1958		167.5	420

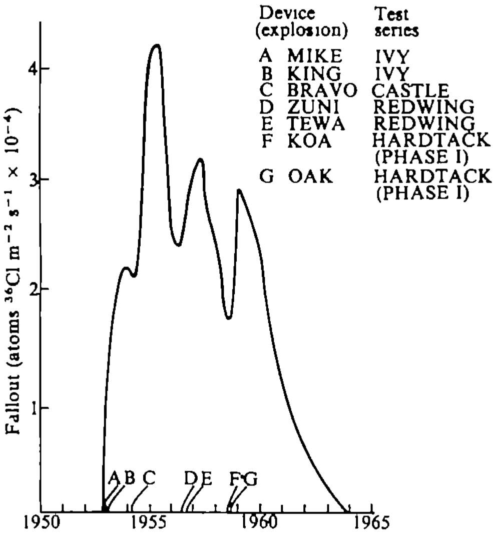

Fig. 1 Fallout of thermonuclear ^{36}Cl predicted by the atmospheric box model.

The model was calibrated using ^{36}Cl concentrations in rainfall on Long Island reported by Schaeffer *et al.*[11]. In five samples of rainwater consisting of composites collected over 2–4 week periods between 1957 and 1960, the mean fallout rate was 2.3×10^4 atoms ^{36}Cl m^{-2} s^{-1} (Table 1). The data show a wide scatter which is typical of radionuclide concentrations in rainfall. The fallout at Long Island calculated from these data was converted to global fallout according to the latitude dependence published by Peterson[9]. The model results using this calibration are illustrated in Fig. 1.

Available data on ^{36}Cl concentrations in modern water are summarized in Table 1. The water samples may be divided into four categories: (1) water collected before nuclear testing, (2) concurrent with testing nuclear devices in and near ocean water, (3) postdating the testing and (4) mixtures from different periods.

The 'pre-bomb' well waters from Spain, Arizona, and Texas have been confirmed as such by ^{3}H and ^{14}C dating but are young enough so that measurable ^{36}Cl decay would not have occurred. ^{36}Cl-to-chloride ratios of these samples conform to the geographical distribution of naturally produced ^{36}Cl fallout predicted by Bentley and Davis[12].

The only undiluted sample of ground water from the period of atmospheric nuclear testing is from a shallow Long Island well reported by Schaeffer *et al.*[11]. The ^{36}Cl concentration of this sample is close to the average for rainfall from the same period.

The San Pedro River sample is probably completely post-bomb, inasmuch as the river is largely ephemeral. The La Jolla and Tucson rainfall samples indicate that the ^{36}Cl content of present-day precipitation has returned to approximate pre-bomb levels. The remainder of the analyses are from hydrological systems which tend to mix water over long time periods, such as lakes, perennial rivers, and recharge areas of aquifers.

Although the data in Table 1 are reasonable in view of the fallout predictions, they yield little information concerning shape and magnitude of the pulse. To obtain more information, we sampled water from the Borden Canadian Forces Base landfill site in Ontario, Canada in November, 1981. The hydrogeology and geochemistry of the site, including the locations we sampled, have been thoroughly investigated[13–15]. The tritium distribution has been investigated by Egboka *et al.*[16]. We sampled vertical profiles through the 'plume' of recent recharge delineated in the cited studies. The ^{36}Cl concentrations of samples collected from nested piezometers T5-A and B and T6-A and B are given in Table 2 and the data from T5 are illustrated in Fig. 2. Piezometer T5 penetrates the centre of the plume, but T6, on the edge of the plume, is not expected to give a representative sampling of the fallout.

Table 2 ^{36}Cl in groundwater from the Borden Canadian landfill site

Piezometer	Depth to sampling (m)	Atoms ^{36}Cl l^{-1} (×10^{-8})
T5A-3	8.84	1.69±0.17
T5A-5	10.97	4.86±0.38
T5A-6	12.03	7.45±1.20
T5A-8	14.14	10.85±0.76
T5A-9	15.24	9.26±0.61
T5B-2	17.00	17.92±1.13
T5B-3	18.29	15.86±1.05
T5B-5	20.00	0.77±0.12
T5B-9	24.00	2.17±0.14
T6A-4	10.97	2.90±0.23
T6A-6	14.02	2.71±0.14
T6A-8	16.76	7.14±0.94
T6B-2	19.51	0.35±0.04

Owing to uncertainties in the flow patterns, the sample depth cannot be definitely correlated with the date of recharge. However, the shape of the pulse is similar to that predicted by the fallout model. Furthermore, the maximum ^{36}Cl concentration is three orders of magnitude above the natural concentration estimated from the precipitation, evaporation, and chloride data of McFarlane *et al.*[13] and Egboka[17] and the geographical distribution of ^{36}Cl/Cl in pre-1953 water estimated by Bentley and Davis[12]. The magnitude of this peak agrees with that of the fallout peak predicted on the basis of Krey and Krajewski's model and calibrated with Schaeffer's data. The maximum fallout rate calculated using the figures from the Borden site is 4.8×10^4 atoms ^{36}Cl m^{-2} s^{-1}, compared with the predicted maximum of 4.2×10^4 atoms ^{36}Cl m^{-2} s^{-1}. The total amount of ^{36}Cl in the vertical profile is 5.75×10^{12} atoms m^{-2}, whereas the total fallout predicted by the models is 7.1×10^{12} atoms m^{-2}.

Some preliminary data have also been obtained from an ice core sample at the Dye 3 site in Greenland[18]. The timing and

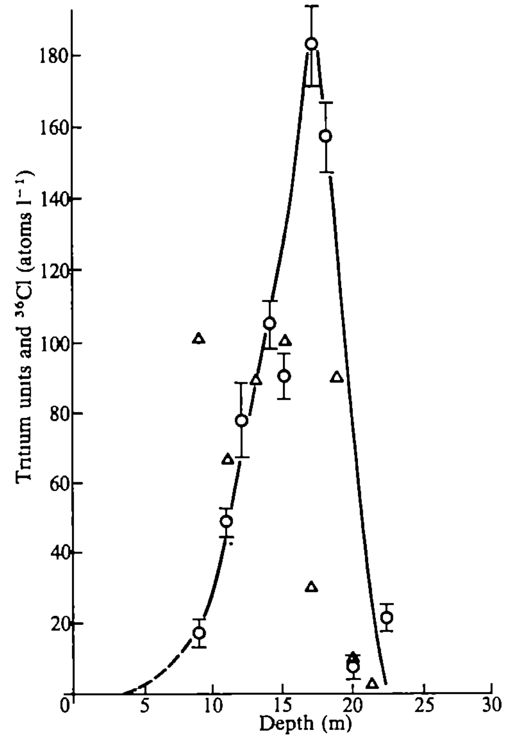

Fig. 2 ^{36}Cl (×10^7, ○) tritium 16 (△, error ±10 tritium units) concentrations in ground water from the Borden Canadian Forces Base landfill in Ontario, Canada. Data for T-5 nested piezometers are plotted.

the general shape of the peak are similar to that shown in Fig. 1, but the maximum concentration is only 80 times the minimum. This contrasts with the 900-fold difference shown in data of Table 1. The contrast could be due to the fact that minimum values measured in the ice may be above background or due to the latitudinal dependence of the fallout. For example, fallout of ^{185}W from the Hardtack series of explosions in the Pacific (which were major ^{36}Cl-producing tests) was more than an order of magnitude greater at 40° N than at 64° N (ref. 1).

We conclude that data obtained thus far are consistent with the ^{36}Cl fallout predictions and that the pulse lasted from 1953 to 1964. In contrast with ^{14}C and tritium, ^{36}Cl fallout rates have returned to levels existing before thermonuclear testing. In the mid-latitudes, the bomb pulse peak height was about 1,000 times the natural background level.

^{36}Cl pulse characteristics appear to be well suited for use as an environmental tracer. The maximum fallout rate was large enough to be unequivocally distinguished from natural concentrations, even after substantial dilution with stable chloride from natural systems. ^{36}Cl is one of the few hydrophilic anthropogenic tracers which has returned to natural levels since the time of the maximum input. This characteristic should be particularly valuable in the study of hydraulic flow and dispersive mixing. Decay of radionuclides and dispersive mixing of ground water create decreases in concentration which are difficult to distinguish. The long half life of ^{36}Cl should eliminate such ambiguities in interpretation of the bomb pulse. We expect that in the near future bomb-produced ^{36}Cl will usefully supplement bomb tritium as an environmental tracer, and that, as the bomb-tritium pulse decays to background levels, ^{36}Cl may eventually replace it.

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The evolution of propagating rifts

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The magnetic survey carried out by Mason and Raff[1,2] off the north-west coast of the US has been central to the development of many of the concepts of seafloor spreading and plate tectonics. Recently Hey and Wilson[3] have again used this survey to illustrate Hey's suggestion[4] that ridge segments sometimes increase their length by propagation. This process leads to a characteristic pattern of magnetic anomalies offset by discontinuities which Hey called pseudofaults because, unlike fracture zones, they (Fig. 1) were never plate boundaries. We demonstrate here how the geometry of the pseudofaults, magnetic anomalies and the failed rift can be worked out using velocity triangles and the concept of stability. This method was originally developed to understand the evolution of triple junctions between three plates[5].

Hey's[4] suggestion is illustrated in its simplest form in Fig. 1*a*. The plate boundary between plates A and B, moving without rotation, consists of two ridge segments normal to the slip vector between the plates and which spread symmetrically with half rate v. The transform fault which joins them is not stationary in a frame of reference fixed to A or B, but moves in the direction shown with a constant velocity V. This behaviour causes rift 1 to increase in length, rift 3 to decrease, and generates a complicated pattern of magnetic anomalies. Though it is possible for ridges and trenches also to move at right angles to their strike, the effects of such motion are geometrically less interesting and are easily analysed. In the case of ridges such motion leads to asymmetric spreading, whereas with trenches it leads either to consumption of both plates or to generation of new plate, presumably by back arc spreading. Whether the real behaviour of a propagating rift will be as simple as that illustrated in Fig. 1*a* is not clear. Analogue experiments with wax[6] suggest that the deformation between the rifts may be distributed, rather than confined to a single boundary, and that rotation may also be important. Such possibilities are, however, ignored here, and the discussion of the evolution is restricted to the simple two plate problem.

Figure 1*b* shows the representation of Fig. 1*a* in velocity space. Since plates A and B both move rigidly they can be represented by two points separated by a distance $2v$. The mid point of the line joining these two points corresponds to one frame of reference in which the ridge axes do not move. Furthermore any frame corresponding to points on the line marked ab$_1$, ab$_3$ passing through this mid point and drawn parallel to the axis also leaves the ridge axes stationary because they are straight. Similarly lines ma and mb correspond to frames in which the magnetic anomalies on plates A and B respectively are stationary. As the transform fault is moving with respect to both plates, it is stationary in any frame corresponding to a point on line ab$_2$, parallel to the line joining A and B but a distance V from it. It is now straightforward to construct the geometry produced by the motions in Fig. 1*a*. Unlike the corresponding problem involving three plates, the two plate system cannot be unstable. The point where the lines ab$_1$, ab$_3$ and ab$_2$ intersect, marked P, corresponds to the frame in which the plate boundary system in Fig. 1*a* is stationary. Hence all points on A will move in the direction PA in this frame, those on B in the direction PB. Hence the traces of the tips of ridges 1 and 3 will be straight lines passing through the ridge tips, parallel to directions PA and PB, shown as dotted lines in Fig. 1*b*. Hey and Wilson[3] refer to the two lines passing through the tip of ridge 1 as pseudofaults and that through the tip of ridge 3 as a failed rift. The geometry of the magnetic anomalies is slightly more difficult to construct. Because the motion of the transform fault is continuous, the region between the pseudofault and the failed rift on plate B must contain continuous anomalies. These are being lengthened by new material added to B from A by the movement of the fault. The magnetic anomalies on plate A and the transform fault are both stationary where the lines marked ma and ab$_2$ intersect at the point M_a in Fig. 1*b*. All points on plate B, including the material added to B by the movement of the fault, must move in the direction of the line M_aB, which is therefore the strike of the magnetic anomalies formed by the transfer of material onto B. The pattern of magnetic anomalies can then be drawn. The resulting

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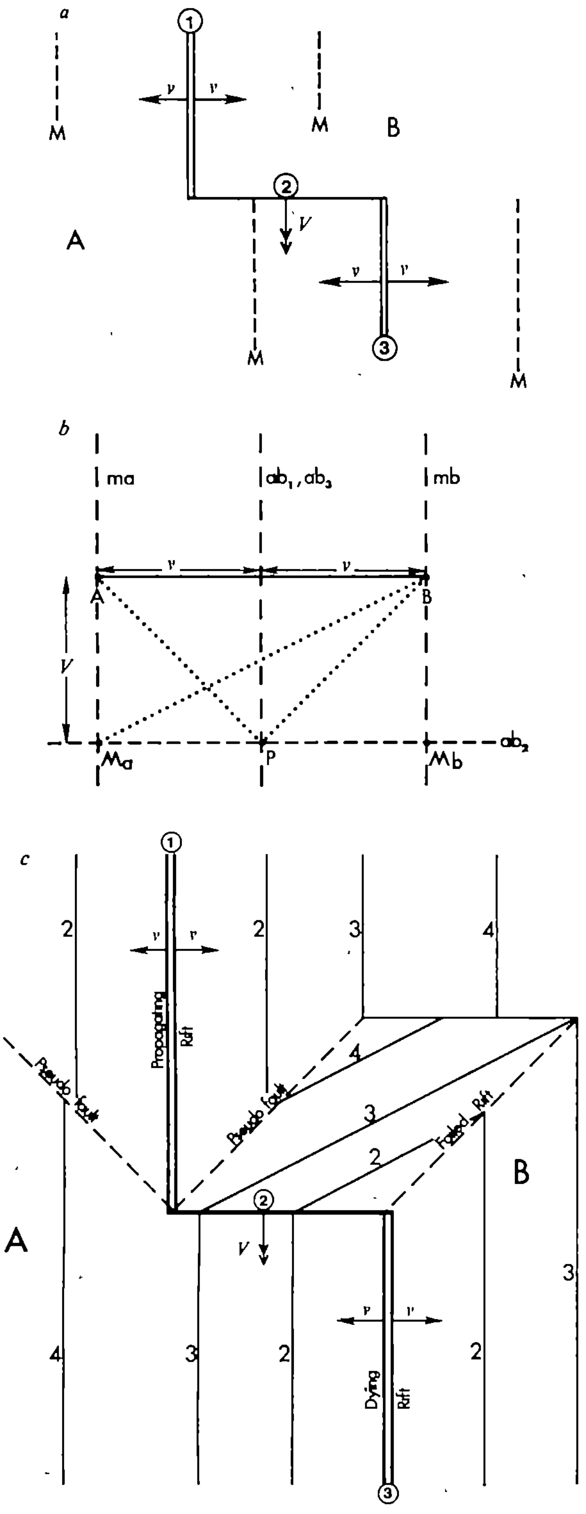

Fig. 1 *a*, Two plates A and B are separating with velocity $2v$ along a plate boundary consisting of two ridges joined by a transform fault. This fault moves with velocity V in the direction shown relative to both plates. *b*, The geometry in velocity space, with dashed lines representing frames in which features remain stationary. For instance any moving frame with a velocity on ma leaves the geometry of the magnetic anomaly M on A unchanged. The pattern of magnetic anomalies produced by these motions is shown in *c*.

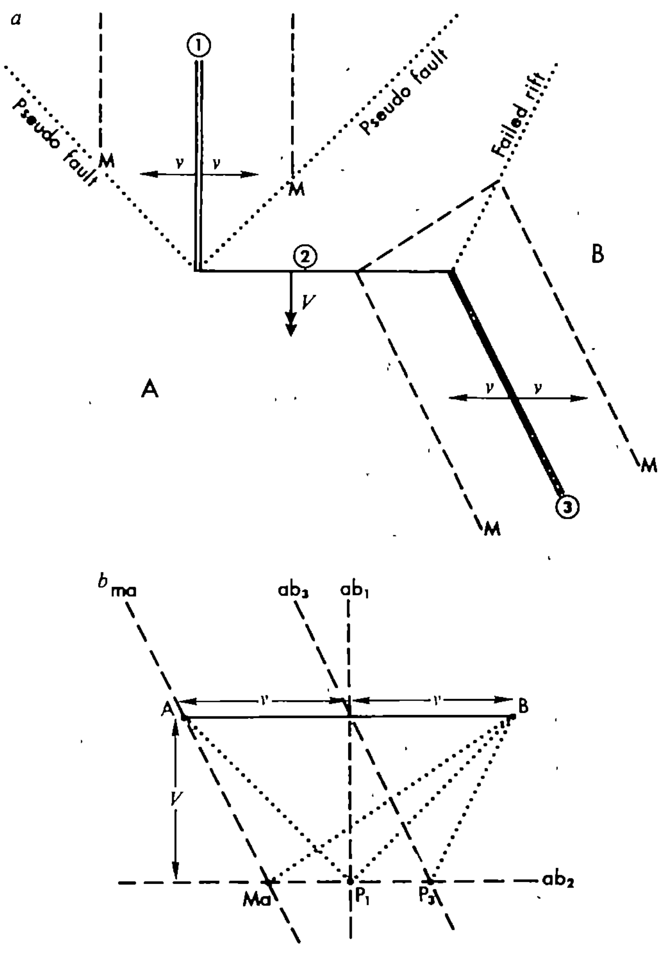

Fig. 2 *a*, As for Fig. 1, but with oblique spreading on one of the ridge segments. *b*, The geometry in velocity space.

arrangement agrees with that shown by Hey *et al.*[7]. Also shown in Fig. 1*c* is the relationship of the anomalies formed by the propagating rift to the anomalies older than anomaly 3 formed by the same rifts when they were not propagating.

Hey's original discussion was concerned with a more complicated geometry than that shown in Fig. 1*a* because one of the ridges involved was spreading obliquely, though still symmetrically. In such conditions it is probable that the ridge which is not spreading obliquely will be the one which propagates, and this geometry is shown in Fig. 2. The evolution may be worked out in exactly the same way as before. Since the two ridges are not parallel, ab_1 and ab_3 intersect ab_2 in different places, P_1 and P_3. Therefore there is no frame in which all three boundaries remain stationary. The tips of the two ridges are separating with a velocity given by the length of the line joining P_1 to P_3, and the pseudofault on plate B is not parallel to the failed rift. Hence the geometry in Fig. 2*a* can be formed by propagation from a ridge axis with no transform fault, provided part of the ridge is spreading obliquely[4]. The strike of the magnetic anomalies produced by the transfer of material from A onto B is parallel to M_aB in Fig. 2*b* as before. Further complications such as asymmetric spreading can easily be included in these constructions.

Hey and his colleagues[3,4,7] have convincingly argued that the magnetic anomalies off the coasts of Oregon and Washington are cut by pseudofaults. What is, however, less clear is whether the anomalies formed by the transfer of material from one plate to another by the movement of the transform fault can be recognized. Detailed surveys of areas where rifts may be propagating are required.

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Gabbroic rocks from the Mathematician Ridge failed rift

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Plutonic rocks from the ocean crust provide direct evidence on the petrological processes leading to its formation and evolution. The nature of such rocks is important for interpreting the origin of on-land ophiolites. So far, oceanic plutonic rocks have been mainly recovered from slow-spreading ridges and their associated fractures[1–13] and from trenches[14,15]. Here we report the results of petrological studies of a suite of gabbros and related plutonic and volcanic rocks dredged from the Mathematician Ridge (MR), which was a fast-spreading ridge (43 mm yr^{-1}) until it was abandoned ~3.5 Myr ago[16]. Textures and mineral chemistry of the rocks together with structural considerations indicate that before abandonment the MR produced fractionated mid-ocean ridge basalt (MORB) and fractionated gabbros. The gradual decrease of spreading rate during abandonment resulted in morphological change, eruption of more primitive MORB and possibly tectonic emplacement of the older ferrogabbros. Alternatively, the latter could have been tectonically emplaced after abandonment.

The MR failed rift consists of a series of short (~50 km) *en echelon* troughs, offset by failed transform faults[16]; its present morphology (Fig. 1) is very similar to that of slow-spreading ridges like the Mid-Atlantic Ridge[17,18] and the Mid-Cayman Rise[19] although magnetic anomalies indicate that before abandonment it was a fast-spreading (43 mm yr^{-1}) ridge[16]. Two dredges of the upper portions of the trough walls (Fig. 1) recovered cumulate gabbro, isotropic gabbro, serpentinized peridotite, diabase, and MORB lavas. Many samples are extremely fresh but most are deformed and metamorphosed or brecciated.

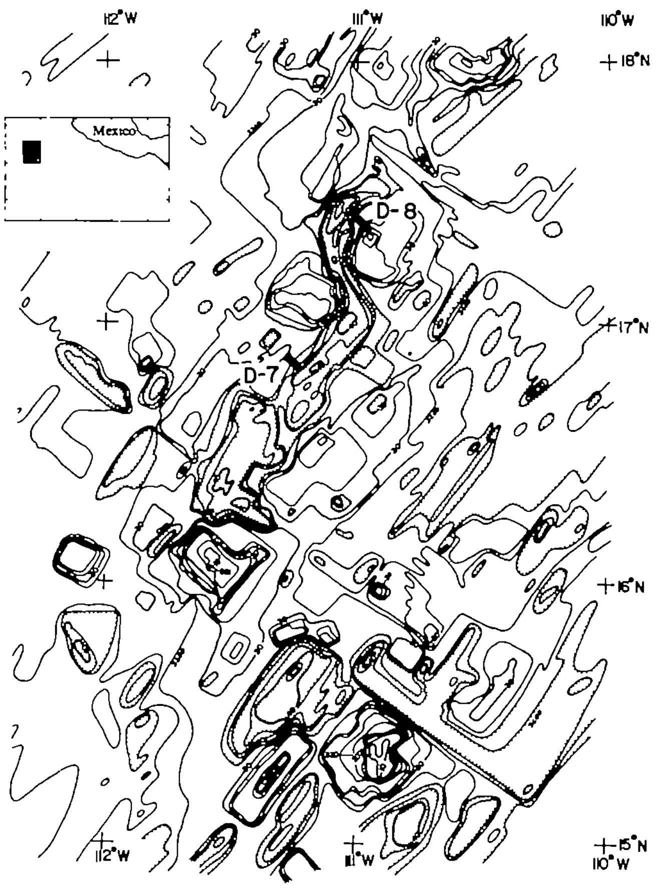

Fig. 1 Bathymetric map (contour interval 200 m) of part of the Mathematician Ridge. Locations of the two dredges that recovered plutonic rocks are shown as solid bars.

Examination of 67 thin sections and microprobe mineral analyses of about 20 representative samples are summarized in Tables 1 and 2 and Fig. 2. The fresh troctolite and other cumulate gabbros have typical cumulate textures, including foliate plagioclase tablets and centimetre-scale mineral layering. Other fresh gabbros have isotropic texture and lack mineral layering. Secondary alteration of these fresh rocks consists only of weak uralitization of pyroxenes (<1%) and very rare

Table 1 Petrographic data for Mathematician Ridge plutonic rocks

Rock type	Troctolite	Cpx gabbro	2-PX Gabbro	Metagabbro	Amphibolite	Breccia
No. of samples	2	21	5	30	4	4
Mode for sample no.	8–7	8–14	8–16	7–1	7–19	8–25
Chemistry for sample no.	8–7	8–14	8–16	7–37	7–43	8–25
No. of points in point count	2,000	2,500	2,500	2,500	2,500	1,500
Olivine (ol)	23% (Fo_{73})					
Orthopyroxene (opx)*	1% ($W_{02}En_{73}Fs_{25}$)		12% ($Wo_{04}En_{66}Fs_{30}$)			
Clinopyroxene (cpx)	16% ($Wo_{44}En_{44}Fs_{12}$)	22% ($Wo_{44}En_{42}Fs_{14}$)	22% ($Wo_{44}En_{41}Fs_{15}$)	11% ($Wo_{46}En_{37}Fs_{17}$)		17% ($Wo_{43}En_{37}Fs_{20}$)
Plagioclase (plag)†	60% (An_{72-60})	78% (An_{67-60})	66% (An_{60-51})	31% An_{34-30})	2% (An_{02})	67% (An_{51-46})
Amphibole (amph)			<1%	34%	80%	15%
Clay				9%	9%	
Opaques	<1%	<1%	<1%	13%	9%	1%
Textures	Fresh cumulate plag laths (<6 mm); interstitial cpx and ol oikocrysts; opx rims on ol	Fresh, plag lath (<6 mm) mildly foliate, with cpx oikocrysts (<20 mm)	Cumulate plag (<7 mm) and opx (<4 mm) with interstitial cpx; slight uralitization	Mylonitic, relict igneous textures, albite veins in plag	Decussate aggregate of hb; mild foliation of opaques; interstitial clay clots	Single- and multi-crystal clasts, subrounded, poorly sorted, cemented by clay-amph matrix
Deformation‡	A	ABC	ABC	CDEF	DF	BG

Accessories not tabulated (all <1%) are epidote, prehnite, quartz, sphene, apatite, analcime; observed exclusively in metagabbro, amphibolite, and breccia, or rarely in late veins through fresh gabbro. Note that as plag becomes more albitic, opx and cpx become more iron-enriched. Metagabbro has cpx similar to that in fresh gabbro, but plag more sodic than in gabbro. Amphibolite has only albite plag, and no cpx. Breccias are inferred to be brecciated gabbro that preserve primary plag and cpx compositions.

* Pyroxene compositions given in terms of molar components wollastonite ($CaSiO_3$), enstatite ($MgSiO_3$), and ferrosilite [(Fe, Mn) SiO_3].

† Plagioclase compositions give range of normal zoning of plagioclase laths. Discontinuous anorthite-rich rims, present in the fresh troctolite and gabbros, are not included.

‡ Deformation styles observed in this rock type A, none except inclusion planes that appear to be healed fractures; B, bent pyroxene lamellae; C, stress-induced plagioclase twins and wavy extinction; D, shear bands, augen, porphyroblasts; E, 'mortar' texture; F, quartz–epidote fracture fills; G, brecciation.

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Table 2 Representative microprobe analyses of pyroxene and amphibole

Mineral	Orthopyroxene	Clinopyroxene	Amphibole		Amphibide		
Rock no.	8–16	8–16	7–43		7–43	7–37	7–37
Analysis no.	(1)	(2)	(3)	1σ	(4)	(5)	(6)
SiO_2	52.92	51.38	43.75	0.76	52 24	44.22	45.28
TiO_2	0.47	0.87	1.42	0.04	0.85	2.22	0.36
Al_2O_3	1.17	2.31	11.84	0.47	4 33	8.76	8.11
Cr_2O_2	0	0.02	ND	ND	ND	ND	ND
FeO^T	19.25	9.03	12.25	0.53	12.14	18 27	24.51
MnO	0.41	0.24	0 13	0.04	0 16	0.26	0 18
NiO	0.04	0	ND		ND	ND	ND
MgO	24.71	14.65	14.03	0.33	16 40	10.73	7.01
CaO	1 28	21.61	11.25	0 13	11.03	10.51	11.85
Na_2O	0.01	0.28	2.29	0 10	1.00	2.55	0.96
K_2O	0	0	0 07	0.01	0 02	0.10	0.07
Cl	ND	ND	0.25	0.03	0 11	0.50	0 12
F	ND	ND	0 01	0.03	0.04	0	0.01
Total	100.26	100 43	97.31		98 32	98.12	98 46
$-O=Cl+F$	0	0	0 06		0.04	0 11	0.03
Total	100.26	100.43	97.25		98.28	98.01	98.43
No. oxygen	6	6	23		23	23	23
Si	1.944	1.912	6 443		7.465	6 667	6.932
Ti	0.013	0 025	0.157		0.091	0.252	0 042
Al	0 051	0.101	2.055		0.729	1.557	1.464
Cr		0.001					
Fe	0.592	0.281	1.510		1.451	2 304	3 137
Mn	0.013	0.008	0.017		0.020	0.033	0 024
Ni	0.001						
Mg	1.353	0.813	3 080		3.495	2.412	1.602
Ca	0 050	0 862	1.777		1.690	1 697	1.945
Na	0 001	0.020	0 653		0.277	0 746	0.286
K			0.013		0.003	0.018	0 014
Cl			0 062		0.027	0 128	0.030
F			0.007		0.022		0.005
Total	4.018	4.023	15.774		15 270	15 814	15 481

Analyses: (1–2): electron microprobe analyses of representative orthopyroxene and clinopyroxene in a 2-PX gabbro (8–16). Other orthopyroxenes in 8–16 show some variation in iron content (up to ±3 mol % Fs component). (3). Average and 1σ standard deviation of eight analyses of a 4-mm hornblende porphyroblast in 7–43 amphibolite. FeO^T represents all iron as Fe^{2+} (4): Core of a small (<1 mm) decussate-textured hornblende in 7–43. Such low-Al cores are zoned to rims that resemble the porphyroblast analysis (3) (5): Brown hornblende from 7–37 metagabbro (6): 0.01 mm thick blue-green amphibole zone that flanks a tiny fracture through the brown hornblende in analysis (5) (7–37) ND, not determined

serpentinite pseudomorphs of olivine crystals. However, the rocks contain evidence of anhydrous subsolidus reactions such as exsolution of pyroxene pairs whose compositions indicate equilibrium temperatures of 896–967 °C (ref. 20) and exsolution of Fe–Ti oxide rods and flakes from plagioclase. Furthermore, plagioclase (An_{82-50}) is often rimmed by discontinuous symplectic anorthite–augite growths or has simple anorthite (to An_{92}) rims (a feature also noted in other cumulates, see ref. 21). Similar textures may form by cotectic crystallization in interstices[21,22], but in Mathematician rocks, small inclusions of anorthite plus pyroxene (either low or high Ca types) plus Fe–Ti oxide occur within plagioclase. This suggests that the anorthite did not crystallize from a silicate liquid.

The olivine and pyroxenes in the fresh gabbros are fractionated (Fe-rich, see Table 1 and Fig. 2) relative to compositions of these minerals in many other oceanic gabbros. They are similar to the most fractionated ones in gabbro from the Mid-Cayman Rise[19], and are more fractionated than practically all the same minerals from DSDP site 334 near the Mid-Atlantic Ridge[4]. The degree of differentiation of the Mathematician magma can be estimated using analysed olivines and Fe–Mg distribution coefficients: If $K_D = 0.30$ (ref. 23) then the range of magma Mg numbers ($Mg/Mg+Fe^{2+}$) was 42–50, based on the range of measured olivine compositions Fo_{71-77}. Olivine compositions from other oceanic gabbros indicate that, if the olivines were cumulus phases and $K_D = 0.30$, the liquids had the following Mg numbers: Mid-Cayman Rise, 41–69 (ref. 19); DSDP site 334, 65–67 (ref. 4); Kane Fracture Zone, 45–67 (ref. 8); Mariana Trench, 49–63 (ref. 15). From the low Mg numbers and the absence of Mg-rich samples (Mg number >50) in the Mathematician suite, it seems probable that these cumulate and isotropic gabbros formed while the MR was spreading rapidly[24–26].

In fresh Mathematician gabbros, the mafic minerals generally become more Fe-rich as coexisting plagioclase cores become

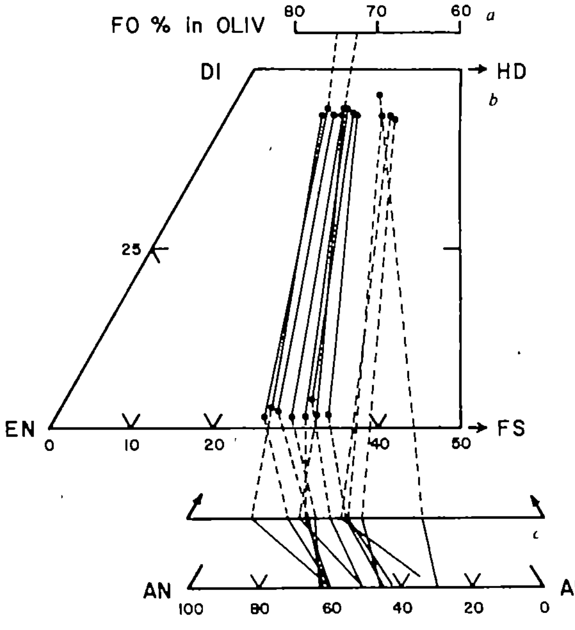

Fig. 2 Plot of electron microprobe analyses of minerals in plutonic rocks. *a*, Average olivine compositions in the two troctolite samples. *b*, Pyroxenes plotted in the conventional pyroxene quadrilateral (molar). The points are average compositions except for two (8–22 and 8–15) which are the most magnesian pairs of a small but detectable (6 mol % Fs component in cpx) intrasample variation. *c*, Molar plagioclase compositions in the Ab–An–Or ternary. Upper ternary represents the most calcic core in the sample, and lower ternary represents the most sodic rim. Discontinuous anorthitic rims (see text) are not plotted Dashed lines connect analyses from the same sample.

more albitic (Table 1, Fig. 2). This suggests that the gabbros originated from multiply-saturated basaltic liquids undergoing slow cooling and fractional crystallization with some crystal settling contributing to the formation of cumulate gabbros.

Most gabbros from the MR are deformed and the extent of deformation is generally correlated with the extent of alteration/metamorphism of primary phases, except in brecciated rocks. These were apparently brecciated by brittle fracturing at low temperature, and contain chiefly clays as secondary minerals. In contrast, most deformed gabbros contain higher temperature metamorphic assemblages (see Tables 1 and 2). A continuous gradation from fresh gabbro to completely amphibolitized gabbro occurs among MR samples. This transformation is characterized by: (1) increase in the abundances of Cl-bearing amphibole (Table 2) at the expense of pyroxenes, and (2) concomitant decrease in the anorthite component in plagioclase: in completely amphibolitized gabbros, the scant plagioclase is albite, which may be retrograde. Additional evidence for retrograde metamorphism in the amphibolitized gabbros includes the presence of epidote (for example, Ps_{15-25} in sample 8-24; Ps_{14} in 8-17), prehnite ($Fe_2O_3 < 0.8$ wt% in samples 8-1, 8-18), albite veins, tremolite ($Trem_{66}Act_{34}$ in sample 8-16, $Trem_{62}$ in 8-24), chlorite, analcime, and quartz and quartz + Ep veins that cut foliation. The quartz veins contain polyphase fluid inclusions which sometimes contain a salt crystal. These inclusions and the Cl content of amphiboles indicate that late hydrothermal fluids were quite saline[11,27].

The serpentinite (82% talc/serpentine, 2% spinel, 16% clay and amphibole) contains Al = rich Cr-spinels [$Cr/Cr+Al = 0.4$; $Fe^{3+}/Fe^{3+}+Al+Cr = 0.01$; $Mg/Mg+Fe^{2+} = 0.64–0.70$] that have very rare inclusions of olivine (Fo_{91}). The spinels form coarse amoeboid grains, similar to a texture inferred by Dick[28] to occur characteristically in peridotite that has been partially melted. By contrast, cumulate spinel grown from a melt is normally euhedral[28]. Therefore, textural evidence, together with the Al-content of the Cr-spinel, suggests that the serpentinitized peridotite represents partially-melted oceanic upper mantle material. Altered diabase recovered in dredge 8 contains secondary albite but relict clinopyroxene ($En_{51}Fs_{11}$) and is relatively unfractionated with $TiO_2 = 1.05$ wt%, Cr = 370 p.p.m., La = 2.68 p.p.m. and $(La/Sm)_N = 0.58$ (ref. 29 and work in preparation). Most of the MORB lavas recovered are also relatively unfractionated. Those from dredge 7 have Mg number = 74, MgO = ~10 wt%, $TiO_2 = 0.72$ wt%, Cr = 500 p.p.m., Ni = ~200 p.p.m., La = 1.0 p.p.m. and $(La/Sm)_N = 0.35$, while the most fractionated one, a MORB lava from dredge 8 has Mg number = 65, $TiO_2 = 1.28$ wt%, Cr = 270 p.p.m.

Plutonic rocks recovered in the MR are broadly similar to other oceanic gabbros, but they comprise a relatively fractionated suite. Detailed differences such as a lack of cumulus spinel, which is present in DSDP site 334 cumulates[4], will be considered elsewhere. The suite of volcanic and plutonic rocks from the MR is similar to that found in ophiolites[30]. However, because both dredges in MR troughs contained all rock types, we have no constraints on stratigraphical thicknesses, or even whether an orderly stratigraphy is exposed. If there is an orderly stratigraphy, then oceanic crust at the MR is very thin (<600 m, from Fig. 1). Otherwise, processes such as tectonic disruption or diapiric rise of serpentinite may have occurred. The chemical contrast between unfractionated MORB lavas and diabase and the relatively fractionated gabbros might suggest that these rocks are in tectonic contact, and gabbroic breccias that formed at low temperature may have resulted from such tectonism. The timing of the presumed tectonic activity is poorly constrained, but some could have postdated the cessation of spreading and the inferred morphological changes associated with gradually decreasing spreading rate of the MR. We favour the notion that fractionated gabbros originated while the MR was spreading rapidly, whereas less fractionated MORB lavas were produced during the transition of the MR to its present slow-spreading morphology, because this is consistent with petrological and geophysical data for presently active slow and fast-spreading ridges[24–26]. Choosing between this hypothesis and the possibilities discussed above will require further detailed studies of the MR and the stratiagraphical/structural relationships of the rocks exposed there.

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Why is Mrs Thatcher interrupted so often?

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If a conversation is to proceed smoothly, the participants have to take turns to speak. Studies of conversation have shown that there are signals which speakers give to inform listeners that they are willing to hand over the conversational turn[1–4]. Some of these signals are part of the text (for example, completion of syntactic segments), some are non-verbal (such as completion of a gesture), but most are carried by the pitch, timing and intensity pattern of the speech; for example, both pitch and loudness tend to drop particularly low at the end of a speaker's turn. When one speaker interrupts another, the two can be said to be disputing who has the turn. Interruptions can occur because one participant tries to dominate or disrupt the conversation. But it could also be the case that mistakes occur in the way these subtle turn-yielding signals are transmitted and received. We demonstrate here that many interruptions in an interview with Mrs Margaret Thatcher, the British Prime Minister, occur at points where independent judges agree that her turn appears to have finished. It is suggested that she is unconsciously displaying turn-yielding cues at certain inappropriate points. The turn-yielding cues responsible are identified.

Mrs Thatcher's interviews display a distinctive pattern—she is typically interrupted more frequently than other senior politicians, and she is interrupted more often by interviewers than she herself interrupts[5]. This pattern is consistent across a wide variety of interviewers (including Denis Tuohy, Brian Walden and Llew Gardner) and across a wide variety of topics[6]. Butting-in interruptions are particularly common in her interviews. These occur where her interviewer interrupts but fails to get the floor, as in the following example where Denis Tuohy (D.T.) tries to take the floor from Mrs Thatcher (M.T.) (*TV Eye*, April 1979).

M.T.: . . . if you've got the money in your pocket you can choose/whether you spend it on things which attract Value Added Tax/or not
D.T.: (You s-
(
M.T.: (and the main necessities don't
D.T.: You say a little on Value Added Tax

where /indicates unfilled pause ≥200 ms

(word 1
(word 2 indicates simultaneous speech

A series of experiments was designed to test the hypothesis that Mrs Thatcher is often interrupted because she displays turn-yielding cues at points where she has not completed her turn. Forty extracts were selected from the Denis Tuohy *TV Eye* interview (ITV, April 1979): 10 were turn-final (that is, utterances at the end of a turn immediately preceding a smooth speaker-switch), 20 were turn-medial (utterances from within a turn), and the remaining 10 were turn-disputed (utterances immediately preceding an interruption by Tuohy). These extracts all contained at least one sentence. The extracts were presented to subjects who had to judge whether Mrs Thatcher's turn was complete or not, in a forced choice procedure. The extracts were presented on video to 79 subjects, on audio only to 29 subjects (in the audio presentation, only the final tone group of each utterance was presented, with two exceptions in which the last two tone groups were played because the final tone group comprised only one word), on vision only to 14 subjects, and typescripts of the extracts were presented to 20 subjects. There were different numbers of subjects in conditions because the extracts were presented in university classes. All subjects were students at the universities of Sheffield or Sussex. For each of the 40 extracts in each of the 4 modes of presentation, the percentages of completion judgements were calculated (see Table 1).

Table 1 Results of completion–judgment experiments to assess the reasons Mrs Thatcher is interrupted so often

Mean percentage of utterances judged to be complete

	Mode of presentation			
	Video	Vision only	Audio only	Typescript
Turn-final	83.530	76.430	62.230	63.500
Turn-disputed	40.120	38.570	55.860	50.500
Turn-medial	23.045	18.570	32.405	58.250

Correlations between presentation modes (% completion judgements for each utterance)

Vision only	Audio only	Typescript	
0.898	0.676	0.083	Video
	0.586	0.000	Vision only
		−0.080	Audio only

An analysis of variance (ANOVAR) revealed that both 'type of utterance' ($F = 23.265$; $P < 0.0001$) and 'mode of presentation' ($F = 8.282$, $P < 0.0001$) significantly affected judgements of completion. There was also a significant interaction effect between type of utterance and mode of presentation ($F = 7.692$; $P < 0.001$). As predicted, turn-final utterances produced the

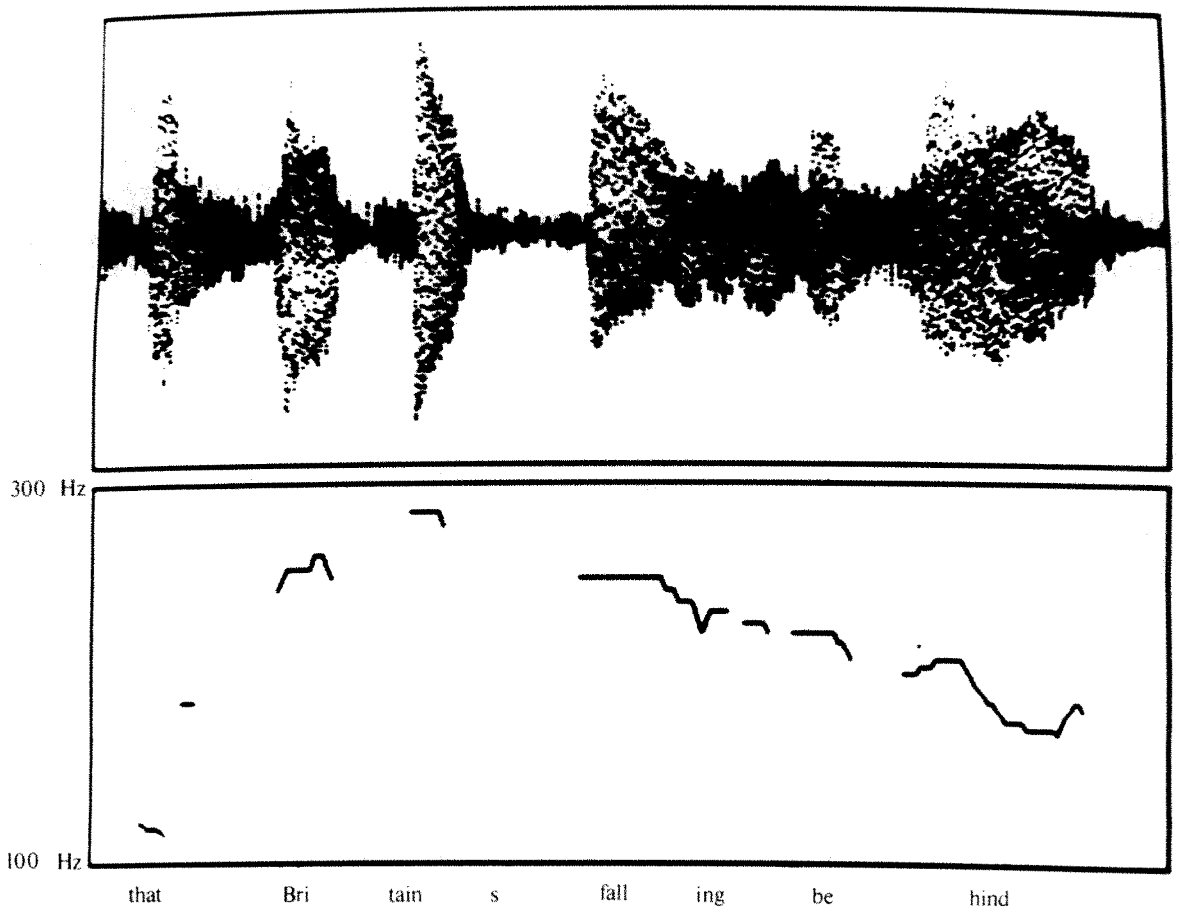

Fig. 1 Amplitude trace and pitch contour of turn-medial utterance, showing slow fall in utterance pitch.

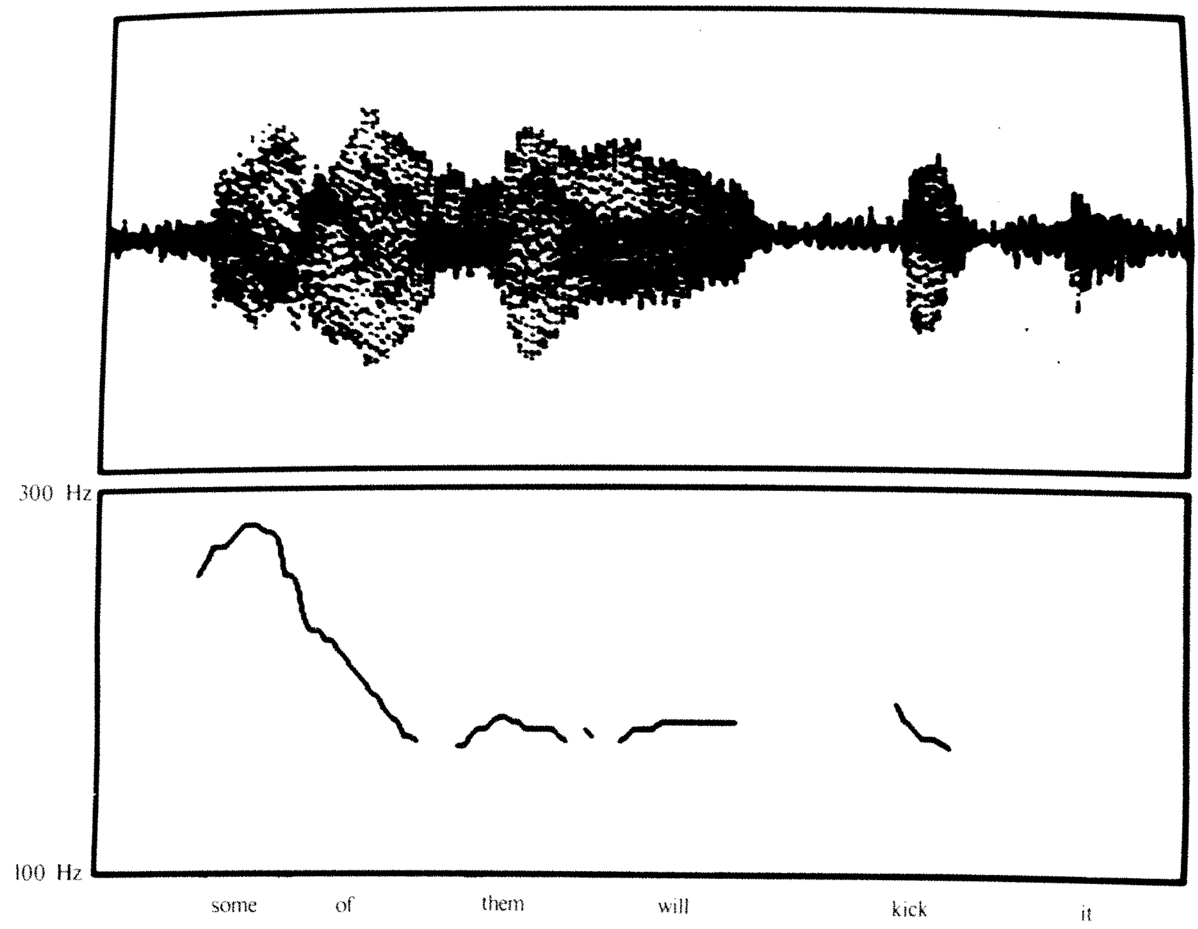

Fig. 2 Amplitude trace and pitch contour of turn-final utterance, showing fast fall in utterance pitch.

most completion judgements, and the turn-medial the lowest, with the disputed turns falling somewhere in between these two. One-factor ANOVARs revealed that 'type of utterance' had a significant effect in the case of the video presentation ($F = 34.791$, $P < 0.0001$), audio presentation ($F = 5.129$, $P < 0.02$) and vision-only presentation ($F = 25.486$, $P < 0.0001$). With the typescript, however, there was no significant effect ($F = 1.185$, not significant). (Note that significant effects did emerge in conditions with smaller numbers of subjects than the typescript condition, thus in fact demonstrating the robustness of the effects.) A comparison of means, using the Tukey HSD procedure (see ref. 12, p. 87) revealed that judged completion scores were significantly higher for turn-disputed utterances than for turn-medial utterances for the video presentation ($F = 5.53$, $P < 0.05$), the audio presentation ($F = 5.15$, $P < 0.05$) and the vision-only presentation ($F = 6.09$, $P < 0.05$).

Tests for correlation revealed significant positive correlations (all at the 0.001 level or beyond) among the video, vision-only and audio-only conditions for proportion of completion judgements for each utterance. The typescript condition judgements, however, did not correlate significantly with any of the other conditions (see Table 1). Thus, although, for example, the means for the turn-final utterances in the audio and typescript conditions are similar, the individual utterance ratings which comprise the means are obviously quite different.

Thus the basic hypothesis was supported—those utterances immediately preceding an interruption by the interviewer were judged to be complete more often than turn-medial utterances and this was not on the basis of syntactic or semantic content (present in the typescript) because, of course, no significant effect emerged in the case of the typescript, but on the basis of information carried in the audio channel and in the visual channel of the interaction, as models of turn-taking such as that of Duncan[2] would predict. Subsequent analyses sought to determine exactly which signals were giving rise to subjects' perceptions of these utterances as complete.

First, the 40 extracts from the Denis Tuohy interview with Mrs Thatcher were digitized and coded into LPC (linear predictive coding) parameters, and a value for the fundamental frequency of each sample was extracted. All 40 utterances terminated in a pitch fall with one exception, a question which ended in a rise; this exception was excluded from further auditory analysis. The value of the fundamental frequency in cycles per second was determined for the highest and lowest points (peak and trough) of each terminal fall. The mean values for the three types of utterance are displayed in the first two columns of Table 2. Column 3 shows the mean pitch range covered by the fall (that is, the difference between columns 1 and 2), and column 4 gives the mean time in milliseconds over which the fall was realized.

ANOVARs on these four measures showed that on two of them, the three conditions differed significantly: (1) The trough of the fall; the difference between conditions was significant ($F = 5.75$, $P < 0.01$), and Scheffé *post-hoc* comparisons showed that the turn-final utterances ended significantly lower than the other two groups, which did not differ significantly from one another.

(2) The timespan over which the terminal fall was realized; the difference between conditions was significant ($F = 4.61$, $P < 0.02$), and Scheffé *post-hoc* comparisons showed that this effect was due to the turn-medial falls being slower than either of the other two types of utterance, which did not differ significantly (see Figs 1, 2). There was no significant difference between the three types of utterance on either the height of the pitch peak or the range of the fall.

This analysis suggests that the turn-disputed utterances may, in fact, have conflicting cues: a fast fall like the turn-final utterances, but a fall which does not descend very low, like the

Table 2 Mean peak and trough values for three types of utterance

	Peak	Trough	Δ	Span
Turn-final	238	141	97	693
Turn-disputed	263	167	96	463
Turn-medial	275	161	114	953

turn-medial utterances. This, in turn, suggests that while the speaker is actually giving a number of cues to the end of her turn, the cue which she considers paramount may be different from the cue which her interlocutor considers paramount. If she considered that her most decisive cue to the end of her turn was letting her voice drop to around 140 Hz instead of keeping it no lower than 160 Hz, whereas her interlocutor considered that her most decisive cue was a rapidly executed final fall rather than a slow fall, their respective decisions as to whether or not she had finished her turn would differ in precisely those cases which were disputed in the present sample of utterances.

One of the authors, trained in phonetic transcription, then transcribed the 40 utterances without knowledge of the position of the utterances in the speaker's turn. The transcriptions of the turn-final and turn-medial utterances were then separately inspected, and the prosodic and vocal quality features occurring in three or more of these utterances listed. Five 'final' features were found: pitch downstep (rapid drop) before the main fall (tonic); double falling contour; allegro portion before the tonic; whispery voice; and creaky voice. Three 'medial' features were found: allegro continuing through the tonic; pitch upstep before the final fall; and a non-falling sustained contour after the fall. (Note that because all our sample utterances ended in falls, we must have underestimated the complete range of cues when non-falling utterances are included. For example, many turn-medial utterances end in a fall–rise.)

The remaining utterances were then checked for the presence of these features, which were assumed to be characteristic of turn-final and turn-medial utterances, respectively. The five 'final' features occurred on average 2.2 times per turn-final utterance, but 0.2 times per turn-medial utterance. Turn-disputed utterances, with a mean of 1.6, were closer to the turn-final utterances than to the turn-medial utterances. The three 'medial' cues occurred on average 0.65 times per turn-medial utterance, but 0.10 times per turn-final utterance. Turn-disputed utterances again fell in between with a mean of 0.30.

The number of acoustic cues present in each utterance was then correlated with the results of the audio perception test, and the proportion of completion judgements for each utterance was found to have a significant positive correlation with the mean number of 'final' cues present in that utterance ($r = 0.46$, $P = 0.003$), but a significant negative correlation with the mean number of 'medial' cues ($r = -0.45$, $P = 0.004$). Thus, the listeners in this experiment seem to have been making use of these phonetic and vocal quality cues in making their forced choice judgements.

In terms of the visual channel, the main signal guiding completion judgements must have been eye gaze because, in a large proportion of instances, the camera angle was such that no information was available on other aspects of nonverbal behaviour such as gesture (gesture termination being an important turn-yielding signal, and gesture maintenance an important turn-holding signal, according to Duncan). Eye gaze has been shown in the past to be implicated in turn regulation[7–9]. Analysis of the video revealed that in 100% of the turn-final utterances, Mrs Thatcher was looking at her interviewer at the end of the utterance, compared with 55% of turn-medial utterances. Turn-disputed utterances were again intermediate with 80%.

We have argued that some interruptions in conversation may arise from the transmission of turn-yielding cues at inappropriate points rather than from any desire on the part of either participant to dominate or disrupt the conversation. It has often been suggested that conversation can be regarded as a highly skilled performance[10,11]. Successful manipulation of these very subtle turn-taking signals must certainly rank as one important aspect of this conversational skill.

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Is annual reproduction in deep-sea echinoderms a response to variability in their environment?

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The deep-sea environment has traditionally been considered as one of the least variable on the surface of the Earth[1–3]. However, recent evidence[4–6] suggests that there may be a seasonal fluctuation in the physicochemical environment, and there is further evidence[7–11] that annual reproductive periodicities may occur among populations of deep-sea invertebrates. We report here evidence for annual periodicities and considerable between-species synchrony in the reproductive cycles of five deep-sea echinoderms, spawning occurring in late winter and early spring. These species also show a similar mode of early development that suggests adaptation to a seasonally varying food supply.

One of the aims of a long-term time series sampling programme initiated by the Scottish Biological Association in the Rockall Trough (north-east Atlantic)[12,13] is to test the hypothesis that annual periodicities may occur in deep-sea invertebrates. In 1975 a permanent station was established at 2,900 m depth (54°40′ N; 12°16′ W). In 1978 this station was supplemented by a second (station 'M') at a depth of 2,200 m nearer the base of the Hebridean Slope (57°18′ N; 10°23′ W). Samples of the benthic fauna have been taken from both stations, using either an epibenthic sledge or an Agassiz trawl at approximately 4-monthly intervals to the present date.

Although a wide variety of deep-sea benthic invertebrates have been collected, echinoderm species dominate in terms of biomass. Seven echinoderm species (Table 1*a*) were collected in sufficient numbers to determine any seasonal reproductive cycles. A further seven species have been examined but were collected irregularly or in insufficient numbers to determine reproductive periodicity (Table 1*b*). In addition, echinoderm material is available to us from a number of other recently obtained samples from the Rockall Trough[14], and fine-meshed rectangular midwater trawl (RMT 1; mesh diameter 0.33 mm) samples have been obtained over station 'M' to look for echinoderm larvae (Table 1*c*).

To investigate the reproductive cycle of each species, the gonads of each specimen were dissected out and processed to paraffin wax, sectioned and stained. In all species examined the sexes were separate and equal in numbers, and since gametogenesis is more easily determined in females, we use data on oocyte size frequencies (Fig. 1*a–c*) to study reproductive seasonality. The diameters of at least 100 oocytes per specimen were measured and from these data oocyte-size/frequency diagrams constructed (Fig. 1*a–c*).

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Table 1 Reproductive biology of deep-sea echinoderms from the northern Rockall Trough

Species	Sample depth (m)	Total no. examined from time-series samples*		Maximum egg size (µm)	Annual reproductive cycle	Ref.
		Female	Male			
a, Species available for examination of seasonality						
Ophiura ljungman	2,900	138	132	120	+	11
Ophiomusium lymani	2,200	180	132	420	Only larval recruitment	13
Bathybiaster vexillifer	2,200	67	51	900	–	15
Plutonaster bifrons	2,200	85	93	110	+	36
Benthopecten simplex	2,200	90	77	900	–	16
Hymenaster membranaceus	2,200	114	104	1,100	–	37
Echinus affinis	2,200	115	121	110	+	(Unpublished)
b, Other species examined						
Dytaster insignis	2,200	6	10	120	?+	36
Psilaster andromeda	2,200	10	12	900	–	36
Pectinaster filholi	2,200	34	31	850	–	16
Pontaster tenuispinus	590–1,050	17†	10†	850	–	16
Pseudarchaster parelii	2,200	7†	6†	900	–	17
Paragonaster subtilis	2,200	6†	15†	900	–	17
Zoroaster fulgens	2,200	34†	20†	900	–	(Unpublished)
c, Larvae of species collected by RMT1						
Ophiocten gracilis	1,000	–		110	+	25

* Immature, indeterminate, hermaphroditic and parasitized specimens are not included.
† Including additional material not from time series.

Among the 14 species of echinoderms (Table 1), two distinct reproductive strategies are evident. In nine of the species, a few, large-sized eggs (~1,000 µm diameter) are produced[15–17]. This is usually interpreted as indicating direct development, omitting a larval stage[18,19]. None of these species show any indication of seasonality in gametogenesis, eggs presumably being produced continuously throughout the year. The production of a few large eggs with considerable reserves of yolk is considered a result of evolutionary selection for increased efficiency of reproduction with minimal wastage of fertilized eggs, embryos or larvae before recruitment to the adult population.

An alternative reproductive strategy is seen in the remaining echinoderms examined (Table 1) and is particularly evident in *Ophiura ljungmani, Plutonaster bifrons* and *Echinus affinis*. To these three species may be added *Ophiocten gracilis* and *Dytaster insignis*. All these species produce a large number of small (~100 µm diameter) eggs which develop as free-swimming larva and feed in the plankton[18,19]. It is of great significance that these echinoderms show both between- and within-species synchrony in egg production (Fig. 1*a–c*). This is especially evident in *O. ljungmani* and *E. affinis*. In the former, gametogenesis is initiated in March/April, and gonad growth continues until maximum development is attained in November. Spent individuals are found in January and February each year. Oogenesis in *E. affinis* appears to start in the germinal epithelium of the ovary at the time of maximum development of the previous year's oogenic growth. The ripe oocytes are spawned in January/February and the newly developing oocytes within the germinal epithelium develop to reach maximum size in November/December, by which time the next cycle of oogenesis has been initiated.

In *P. bifrons* the annual oogenic cycle does not appear as discrete as that of *O. ljungmani* and *E. affinis*. Gametogenesis can occur from January to at least May. There is a period of oocyte growth before spawning, which appears to occur from November to March.

The degree of variation in gonad development, both between samples and between individuals within samples, may be determined by the analysis of oocyte size/frequency data using a mixed model, nested-design, analysis of variance[20]. The percentage of total variance, and the variance between individual

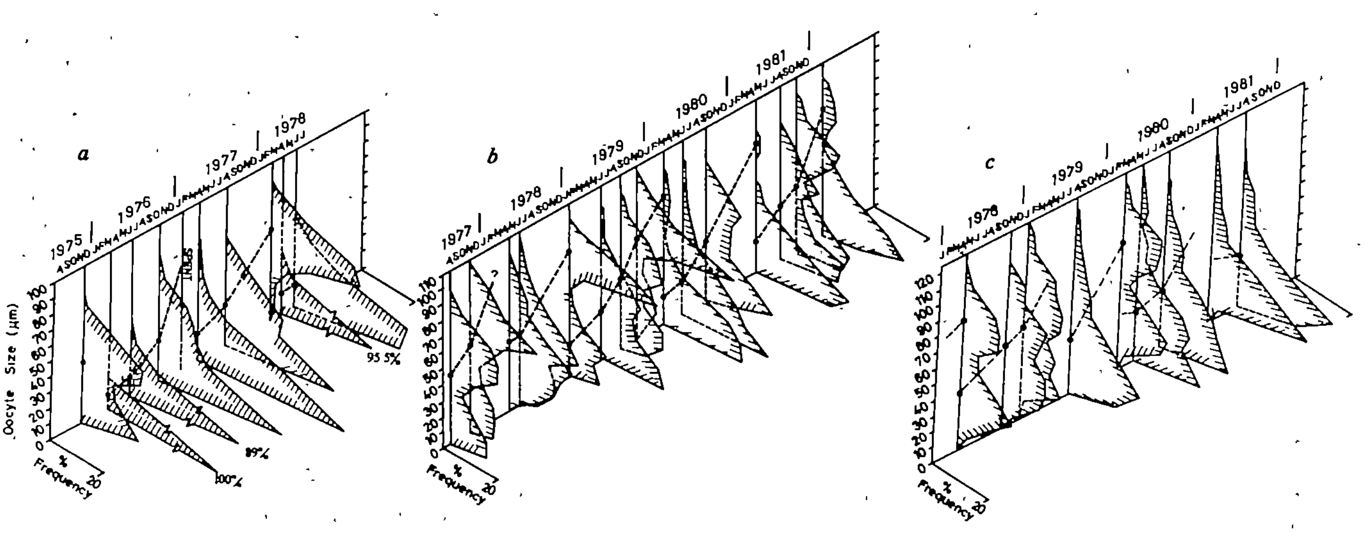

Fig. 1 The percentage frequency distribution (abscissa) for different oocyte sizes (ordinate) for each sample against time. *a*, *Ophiura ljungmani*. *b*, *Echinus affinis*. *c*, *Plutonaster bifrons*. ● Represents mean oocyte diameter for each sample.

Table 2 The degree of gonad development in deep-sea and shallow-water echinoderm species

Species	Between samples*	σ_s^2 % Variance between samples	σ_I^2 % Variance between individuals	% Variance within individuals	j $100\times\sigma_s/\sigma_s+\sigma_I$
Echinus affinis	21.2316 (11)	20.98	9.53	69.49	68.75
Plutonaster bifrons	9.4108 (12)	18.31	11.87	69.82	60.68
Ophiura ljungmani	22.1720 (13)	45.01	22.55	32.45	66.63
Bathybiaster vexillifer	3.3386 (9)	2.10	7.48	90.43	21.89
Benthopecten simplex	0.9261 (8)	0.0	21.82	78.18	0.0
Hymenaster membranaceus	3.5355 (10)	1.96	6.55	91.49	23.03
Ophiomusium lymani	4.6488 (16)	3.94	7.86	88.20	33.40
Ophiura albida†	18.0783 (20)	27.19	13.11	59.70	67.48
Ophiura texturata†	5.2900 (19)	11.24	20.64	68.12	35.25

* Value in parentheses indicates degree of freedom in numerator of *F*-ratio; degrees of freedom of denominator are effectively ∞.

† *Ophiura albida* and *O. texturata* are shallow-water species included for comparison. j = % of variance between individual means explained by sample means.

mean oocyte size accounted for by the sample means is large (>60%) for *O. ljungmani, E. affinis* and *P. bifrons* (Table 2). These data are comparable with values for shallow-water species known to breed seasonally (Table 2)[21]. Highly significant variation in mean oocyte size occurs between samples within each species. By contrast, although there are significant differences in sample means for *Bathybiaster vexillifer, Hymenaster membranaceus* and *Ophiomusium lymani,* this accounts for a much smaller percentage of variance between samples (Table 2). In *Benthopecten simplex,* the sample means are not significantly different.

The values suggest that there is a distinct variation in mean oocyte size throughout the year in *E. affinis, O. ljungmani* and *P. bifrons* and confirms the observations of seasonal variation seen in the oocyte size/frequency figures.

These data indicate an annual reproductive periodicity that culminates in a spawnout of gametes in January/February each year. In addition to the evidence of seasonal reproductive cycles shown in gametogenic cycles of echinoderms, the occurrence of larvae has been followed in seasonal hauls using the RMT 1. The larval form, *Ophiopluteus ramosus*, was taken abundantly only in the spring (April to June) of each year. The spring-time occurrence of this larva has been noted for several years[22–24]. *Ophiopluteus ramosus* is now known as the larval form of *Ophiocten gracilis*[25]. This brittle star has a distribution centred on 1,000 m, where we may conclude it also shows an annual reproductive cycle.

In addition to the reproductive data, the epibenthic-sledge time series has provided evidence of a late spring/summertime recruitment of post-larvae of both *O. ljungmani* and *E. affinis*[11,26].

From these data we see that four species of deep-sea echinoderm, *O. ljungmani, O. gracilis, P. bifrons* and *E. affinis*, have highly synchronous reproductive cycles. We must infer that some exogenous factor(s) control synchrony of spawning and act as the ultimate selective factor determining reproductive seasonality[27].

Recent discoveries of seasonal periodicities in the physical environment and in sedimentary organic flux from the surface within the deep sea[4–6] suggest environmental factors have a role in the control of reproductive seasonality in deep-sea populations. The reproductive cycle of benthic invertebrates must be controlled at several levels: (1) initiation, (2) gamete development, (3) spawning, and (4) the period of larval development. Factors controlling both initiation of gametogenesis and gamete development are difficult to define[28] even in shallow-water species, although energy available as food will be a significant controlling factor for vitellogenesis. In shallow water, spawning is known to be precipitated by changes in the physical environment such as seasonal temperature fluctuations. Although such changes have been considered imperceptible in the deep sea, recent long-term measurements of current velocity show oscillations of varying period from semidiurnal and diurnal tidal components to low frequency signals, with seasonal maxima in late winter and minima in summer, in surface-generated kinetic energy[6,29]. Although rapidly diminished with depth, the seasonal effects of such kinetic energy (that in the Rockall Trough perhaps are associated with travelling disturbances created by mixing of Atlantic and Norwegian Sea water[29]) may be transmitted to abyssal depths well exceeding that of the 2,900 m deep Permanent Station[6]. Time-lapse photographs have shown that the behaviour of deep-sea fish may be influenced by semidiurnal tidal currents in the Bay of Biscay[30]. However, the possible role of low-frequency physical periodicities as proximate cues in triggering spawning of ripe echinoderms remains speculative. Possibly, secondary effects such as periodic sediment resuspension above critical erosive current velocities at the benthic boundary[31] are more important than direct effects of water motion on echinoderms lying on the seabed. However, chance, isolated triggering of spawning of an individual may be more rapidly transmitted throughout a ripe population by chemical signals in times of high water motion than in quieter periods.

Of considerably greater significance is the inference that in all the seasonally-reproducing deep-sea echinoderms, larvae are produced from February to June, coinciding with the springtime maximum in phytoplankton production in this area of the north-east Atlantic[32]. We believe that there is a sufficiently rapid transfer of small organic particles resulting from surface production, to the deeper water where, together with its associated bacteria, it would be available as food for the free-floating planktotrophic larvae. There is considerable evidence that this transfer of surface production to deep water is both rapid[33–35] and highly seasonal[4,5]. Such a cycle in the availability of surface-derived food material would be expected to influence the evolutionary selection for annual reproduction; larvae produced outside the period of elevated food supply being unlikely to survive.

Although we believe that this hypothesis is valid for deep-sea echinoderms with annual reproductive cycles, we have yet to determine whether such factors determine the reproductive strategies of other deep-sea invertebrates.

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Analysis of the dual role of phytochrome in the photoinhibition of seed germination

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Light can both promote and inhibit germination, even of seeds of the same species[1–3]. Many seeds considered to be positively photoblastic are inhibited by prolonged irradiation with light of a wide variety of spectral qualities, including those, such as sunlight, that promote germination when given as a short exposure[4]. The promoting effect of light is mediated by the red/far-red reversible receptor pigment phytochrome operating in its P_{fr} form. The nature of photoinhibition is much less clear, although it is possible that continuous excitation of phytochrome is involved[5]. It has been suggested that light could control photomorphogenesis in seedlings not only through the establishment of a particular amount of phytochrome in its active P_{fr} form but also by determining the rate of 'cycling' between P_r and P_{fr} forms[6,7]. Such effects of prolonged irradiation are both wavelength and fluence rate dependent. We show here that the photocontrol of seed germination in *Sinapis arvensis* L. involves a promoting reaction dependent on the proportion of phytochrome in the P_{fr} form at photoquilibrium (ϕ) and an inhibiting reaction dependent on the rate of phytochrome interconversion or 'cycling' (H). Thus percentage germination can be quantitatively predicted from the spectral quality and the fluence rate of the seed's light environment.

The photoinhibition of seed germination under prolonged light does not show reciprocity (dependence on the product of fluence rate and exposure time), the length of the irradiation period being an important factor independently of total fluence. The action peak of the response occurs at about 710–720 nm in the few species studied, such as *Lactuca sativa* L.[7,8] and *Poa pratensis* L.[9]. This is characteristic of the 'high irradiance reaction', most studied in seedling photomorphogenesis[7,10,11]. However, in seed germination the high irradiance reaction is antagonistic to the low energy inductive reaction, whereas in seedling photomorphogenesis the two processes are synergistic.

The light reactions of phytochrome can be summarized in the following way:

$$P_r \underset{k_2\,(=N\sigma_2)}{\overset{k_1\,(=N\sigma_1)}{\rightleftarrows}} P_{fr}$$

where N is the photon fluence rate, and σ_1 and σ_2 are the photoconversion cross-sections. Light establishes a photoequilibrium between P_r and P_{fr}, expressed as the proportion of total phytochrome (P) in the form P_{fr}, ϕ. If a constant amount of phytochrome is assumed (there is very little evidence for P_r synthesis or P_{fr} destruction in seeds, although it is difficult to make direct measurements on phytochrome in the tissue of small seeds), then ϕ will be proportional to the amount of P_{fr} provided that the thermal reversion of P_{fr} to P_r is slow relative to the light reactions. However, this represents a dynamic condition of pigment interconversion, both reactions occurring simultaneously although with different rate constants. Thus for any given light condition there is a characteristic 'cycling rate' or 'photostationary flux'. The 'relative' cycling rate is here designated as H. Both ϕ and H can be calculated from the spectral photon distributions of the light source, using published[12] values of the phytochrome photoconversion cross-sections (σ_1 and σ_2) and the following equations:

$$\phi = \frac{\Sigma N_\lambda \sigma_{1\lambda}}{\Sigma N_\lambda \sigma_{1\lambda} + \Sigma N_\lambda \sigma_{2\lambda}}$$

$$H = (1-\phi)\Sigma N_\lambda \sigma_{1\lambda} = \phi\, \Sigma N_\lambda \sigma_{2\lambda}$$

It is also possible to estimate ϕ and H for a particular light source by spectrophotometric measurements on dark grown seedling tissue, which is rich in phytochrome. Tissue is exposed to the light source for increasing periods of irradiation, and the

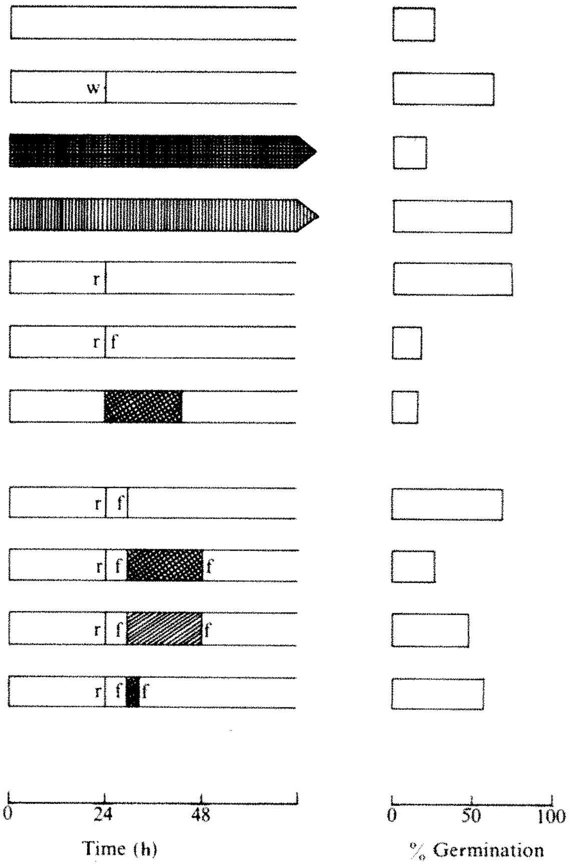

Fig. 1 Photocontrol of seed germination in *Sinapis arvensis*. Seeds irradiated and incubated at 15 °C. Treatments are indicated as follows: r = 5 min red light; f = 15 min far-red light; w = 15 min white incandescent light. ☐, Dark; ■, high fluence rate white incandescent light; ▥, low fluence rate white incandescent light; ▩,high fluence rate light, $\phi = 0.27$; ▨, low fluence rate light, $\phi = 0.27$.

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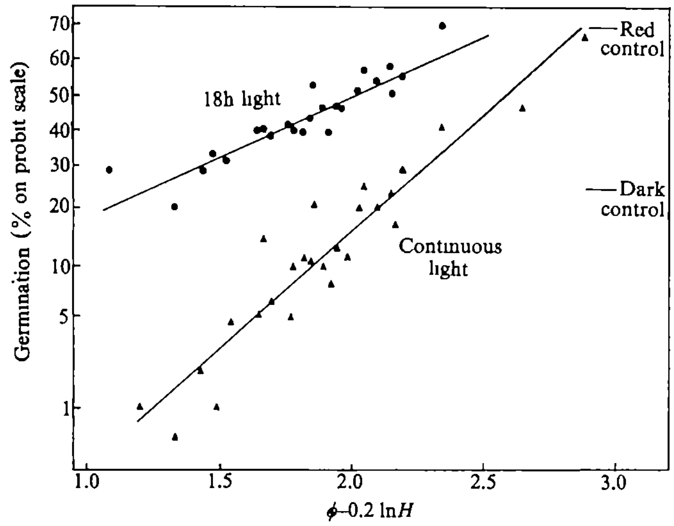

Fig. 2 Germination of *Sinapis* seeds in light of varying ϕ and H at 15 °C. Seven values of ϕ were used ranging from 0.05 to 0.70. Each point represents the mean percentage germination of four dishes of 50 seeds each. Standard errors were in the range 1 to 4, the average being 2. These were placed in a crossed gradient consisting of five 1.5 m 65/80W cool white (daylight) fluorescent tubes (with half their length blacked out) at one end (establishing a high ϕ value), and a 1-kW incandescent spotlight at the other end. The spotlight was placed behind a filter consisting of 10 cm of water, and one layer of no. 5A deep orange and one of no. 20 deep blue primary Cinemoid, which removed practically all irradiation below 700 nm and thus established a low ϕ value. Seeds were exposed either continuously from the start of imbibition until final germination was reached (▲), or as part of the following regime: 24 h dark – 5 min red – 5 h dark – 15 min far-red – 18 h treatment – 15 min far-red – 3 d dark (●). ϕ and H were determined by spectral measurement using a modified ISCO model SR spectroradiometer with a cosine-corrected remote probe. Spectrophotometric measurements of the P_{fr}/P ratio were made on dark-grown *Avena* coleoptiles which were kept on ice throughout exposure to the light. The coleoptiles were then packed into a specially designed cuvette under green safelight, and transferred to a Hitachi/Perkin-Elmer 156 digital dual wavelength spectrophotometer with a special actinic irradiation accessory.

proportion of phytochrome as P_{fr} measured in each case. ϕ is the P_{fr}/P ratio after a saturating irradiation. H can be calculated from ϕ and the time ($t_{\frac{1}{2}}$) to reach the half-saturation value of P_{fr}/P. For the reaction $P_r \rightarrow P_{fr}$:

$$k_1 = N\sigma_1 = \frac{\ln 2}{t_{\frac{1}{2}}}\phi$$

$$H = (1-\phi)k_1$$

$$= \frac{\ln 2(1-\phi)\phi}{t_{\frac{1}{2}}}$$

In this study ϕ and H were varied by setting up a crossed gradient with two light sources at opposite ends of a constant temperature (15 ± 1 °C) growth room. There was a low ϕ value at one end of the room, and a high ϕ value at the other end. ϕ and H were determined at several positions along the gradient, both by spectral measurement and spectrophotometric measurement on coleoptile tips. The two independent methods of determining ϕ and H gave very similar values.

Thus ϕ and H can be used to describe the light environment in terms of the photoreactions of phytochrome. These values may not necessarily reflect the state of phytochrome within the seed, as this is affected by the transmission characteristics of the seed coat and internal tissues.

The photocontrol of *Sinapis* seed germination at 15 °C is summarized in Fig. 1. This shows the normal red/far-red reversible germination response, with a short far-red treatment inhibiting germination below the dark level. An 18-h period of high fluence rate light which establishes a ϕ value of 0.27 will reduce germination below this far-red level. If a 5-h dark period is given between red and far-red pulses, the seeds escape far-red reversion. This indicates that P_{fr} action, whatever this may entail, has been completed. However, it is still possible to block the product of P_{fr} action by giving an 18-h period of high fluence rate light after the escape from reversion, and low fluence rate light has a blocking effect as well, although reduced. The data show that the photoinhibition process is both time and fluence rate dependent.

There appears to be two processes controlling *Sinapis* seed germination. P_{fr} is essential for an early event in the processes leading to germination. However, a time- and fluence-rate-dependent process will block germination at a stage after the apparent completion of P_{fr} action. These two processes can thus operate sequentially, which can lead to the loss of photoinduction so that the P_{fr} promoted process must be repeated. Thus in the natural light environment the two processes will operate simultaneously and antagonistically.

Seeds were sown in dishes along the crossed gradient to examine the relationship between germination and the two phytochrome parameters, ϕ and H. H was varied by shading the seed dishes with muslin or Cinemoid neutral density filters. The prolonged irradiation period was given both after the completion of P_{fr} action and continuously from the start of imbibition. It was found empirically that for a given ϕ value, the probit of germination was inversely proportional to the logarithm of H, and that the proportionality constant was ~0.2 for all ϕ values in both types of experiment. The straight line relationship between a mathematical expression combining ϕ and H and subsequent germination is illustrated in Fig. 2. This shows the antagonism between ϕ and H in the events leading to germination. From this relationship it is possible to predict the percentage germination of *Sinapis* seeds from a description of the light environment in terms of ϕ and H.

A theoretical 'action spectrum' can be generated from this relationship by choosing a particular level of response and calculating the value of H which will give this response for monochromatic irradiations across the spectrum. The absolute photon fluence rate required at each wavelength to achieve this response can be back-calculated from H. The reciprocals of these values when plotted against wavelength produce the action spectrum shown in Fig. 3. This should only deviate from an experimental action spectrum in those parts of the spectrum where the rate of P_{fr} dark reversion is appreciable compared with the rate of the photoreactions.

The striking point about this generated action spectrum is its similarity to experimental action spectra published for the high irradiance reaction in seeds[7,9]. It is therefore reasonable

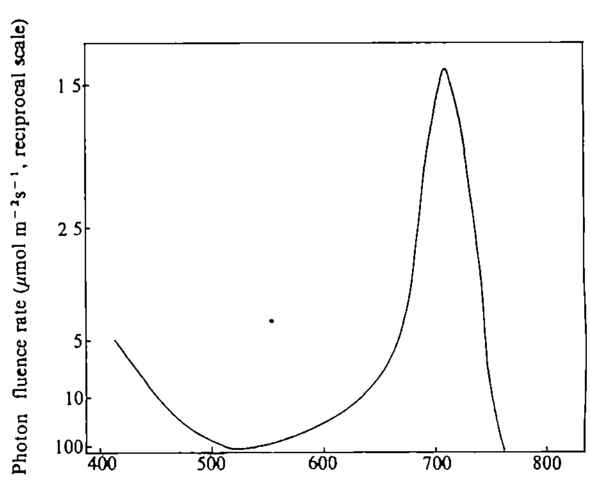

Fig. 3 Relationship between wavelength and calculated photon fluence rate giving half-maximum germination in *Sinapis* seeds. These were calculated using the expression $\phi - 0.2 \ln H = 1.93$, the latter being obtained from the data for 18-h irradiations given after escape from reversion.

to assume that these action spectra (strictly speaking these data for wavelength dependence are not true action spectra) arise because of the interaction of two aspects of phytochrome, the photoequilibrium ϕ and the photoconversion rate H. Further unpublished experiments using the seeds of *Plantago major* L. and *Bromus sterilis* L. support this view. Seeds of *Bromus sterilis* are apparently unique in that P_{fr} actually inhibits germination[13], and experiments using prolonged irradiations with this species suggest that P_{fr} and H will interact such that both processes inhibit germination.

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Regeneration of normal and fertile plants that express octopine synthase, from tobacco crown galls after deletion of tumour-controlling functions

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Crown gall, a neoplasmic transformation of plant cells, is caused by transfer and integration into nuclear plant DNA of T-DNA, a segment of the Ti plasmid of *Agrobacterium tumefaciens*[1–8]. In plant cells, this T-DNA is expressed[9–12] and determines the morphogenetic growth properties of crown gall cells[12–16] and the synthesis of opines[17–21]. The T-DNA in octopine crown galls is composed of either one or two fragments derived from a continuous Ti sequence. Thus far, all octopine tumour lines contain the TL fragment (14 kilobases (kb)), whereas TR-DNA (~6 kb) can be present as an additional sequence[22–24]. Foreign DNA, inserted in the T region of a Ti plasmid, is co-transferred to the plant genome[17,18,21]. However, an important requirement for the use of T-DNA as vectors for plant genetic engineering is the regeneration of normal plants which stably maintain, express and sexually transmit the transferred DNA. We report here the isolation, from tumour tissues induced by mutant Ti plasmids, of morphologically normal plants that produce octopine synthase in all tissues. This enzymatic activity is inherited as a dominant mendelian marker[25]. These regenerated plants retained and expressed only the octopine synthase gene of the T-DNA.

On *Nicotiana tabacum*, *Agrobacterium* strains harbouring the mutant Ti plasmids, pGV2100 (ref. 15) and pGV2206 (ref. 21), induced small, greenish tumours covered with numerous shoots, whereas infection with the wild-type octopine plasmid resulted in large, white tumours without visible differentiation. Bacterium-free tumour tissue cultures induced by these mutant Ti plasmids proliferate on hormone-free medium at the same rate as wild-type tumour cells and they synthesize octopine. The greenish aspect and the ability to form shoots were maintained after repeated subculturing.

In an initial experiment 250 shoots from 9 independent tumour lines induced by pGV2100 were tested, and one was found to synthesize octopine. This plant, rGV1 (ref. 25), was derived from tumour line lGV20 and developed normal roots on culture on hormone-free Murashige and Skoog[26] medium.

Out of 250 shoots, from 8 independent pGV2206 tumour lines incited on tobacco and growing at 25 °C, no octopine-positive shoots could be recovered. Teratomata formation by tumours induced by nopaline strains on tobacco was increased by incubation at 33 °C. Under these circumstances both normal shoots, able to form roots after separation from the tumour, and teratoma-like shoots unable to form roots were observed for the pGV2206-induced tumour lines. From these tumour lines 250 shoots were derived at 33 °C and analysed for the presence of octopine. One tumour line, lGV23, produced 4 octopine-positive plants in 54 shoots tested. Like rGV1, these regenerates, rGV2, rGV3, rGV4 and rGV5, were morphologically normal and could be transferred to soil. Physical analysis of the regenerates by Southern blotting hybridization revealed that they were identical (G. Gheysen, personal communication).

The octopine-positive regenerates obviously differ from suppressed nopaline teratoma shoots obtained from T37 nopaline tumour lines[27–30] in the following aspects. (1) The shoots readily form a normal root system. (2) The shoots develop into plants which do not form spontaneous tumours and their cells are strictly dependent on exogenous hormones for division. (3) The regenerated plants respond to infection with agrobacterial strains carrying either wild-type or mutant Ti plasmids in an identical way to wild-type tobacco. (4) Earlier data, obtained with rGV1, showed that flower morphology and fertility were normal and that opine synthesis is completely retained through the meiotic process[25].

To determine whether all cells of the rGV4 express the octopine synthase gene, protoplasts were prepared from leaf mesophyll cells[25]. Fifty independent calli were tested and found to be positive for octopine synthase activity[31]. To test whether the T-DNA inserts were at identical or different chromosomal loci in rGV1 and rGV4, these plants were crossed and the offspring was analysed. Using a semiquantitative LpDH assay[23] a number of offspring plants were identified presumably containing both T-DNA inserts. Two of these plants were castrated and fertilized with pollen from wild-type W38-tobacco. In one case 71% of the plantlets and in the other case 76% were found to be octopine-positive. These results indicate that the T-DNA inserts in rGV1 and rGV4 are genetically unlinked.

The T-DNA-derived transcripts in the regenerated plants and in the parental tumour tissues were compared with those present in the wild-type octopine tumour line A6-S1 (Fig. 1). The pGV2100-induced tumour line lGV20 clearly lacks transcript 2, and contains a very reduced level of transcript 1 (Fig. 1). The pGV2206-induced tumours lack both transcripts 1 and 2 and contain normal levels of the other TL transcripts[16]. In contrast, rGV1 only expressed transcript 3, which specifies octopine synthase[20] (Fig. 1). Four lGV23-derived regenerates, rGV2, rGV3, rGV4 and rGV5, were also found to express transcript 3 only, as ^{32}P-labelled cDNA, made towards their poly(A)$^+$ RNA, hybridized solely with the T-DNA region overlapping with pTiB6S3 fragments *Sma*I-3b and *Eco*RI-7 (data not shown). Thus, the octopine synthase gene is the only TL-DNA gene that remained expressed in the octopine-positive plants.

We analysed the T-DNA sequences in lGV20 (tumour line) and in rGV1 (regenerated plant) by DNA/DNA hybridizations. These revealed that the T-DNA in lGV20 is co-linear with the T-region of pGV2100. In rGV1 DNA, however, only probes from the right end of the TL-DNA hybridized and revealed a single 3.2-kb fragment in a *Hin*dIII digest (Fig. 2). This indicated that secondary rearrangements have deleted most, if not

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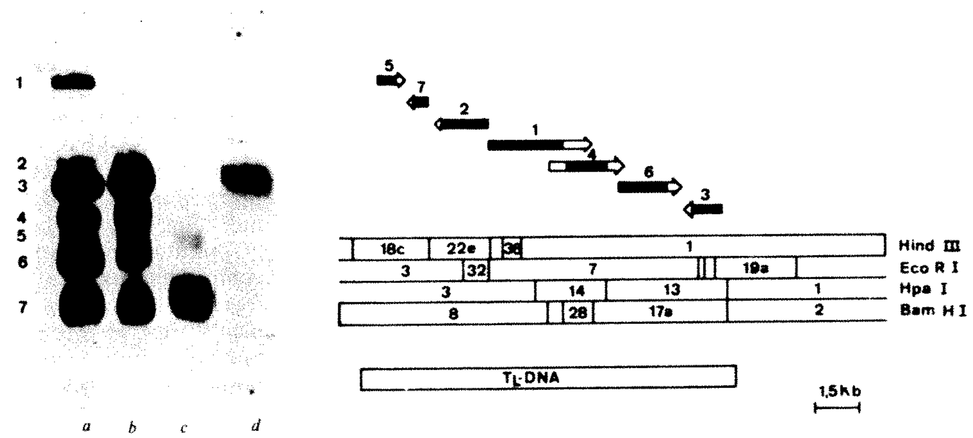

Fig. 1 Analysis of T-DNA transcripts. Total RNA isolation and selection of poly(A)$^+$ RNA fraction from leaves of rGV1, from callus of pGV2100-induced tumour line lGV20 and from the A6-S1 suspension culture was according to ref. 10. Electrophoresis in 1.2% agarose after denaturation with glyoxal/dimethyl sulphoxide, transfer to diazobenzyloxymethyl paper, pretreatment of the filters and hybridization to ^{32}P-labelled T-DNA probes were performed as described elsewhere[12]. Hybridization of poly(A)$^+$ RNA from wild-type octopine tumour line A6-S1 against total T-region probe (a mixture of nick-translated pTiB6S3 *Bam*HI fragment 8 and *Hin*dIII fragment 1) shows the seven TL-encoded transcripts (numbered 1 to 7 according to diminishing size) (lane *a*). Hybridization of poly(A)$^+$ RNA from lGV20 against total T-region probe (lane *b*) and against *Bam*HI fragment 8, which contains the complete coding sequences for transcripts 5, 7, 2 and part of the coding sequences for transcript 1 (lane *c*), clearly indicate the absence of transcript 2, a very reduced level for transcript 1 and wild-type levels of transcripts 5, 7, 4, 6 and 3. Hybridization of poly(A)$^+$ RNA from rGV1 against total T-region probe only lights up transcript 3 (lane *d*). The location of the seven TL-encoded transcripts on the physical map of the pTiAch5 T-region is from ref. 12. The direction of transcript 5 was determined from sequence data (J. Gielen, personal communication).

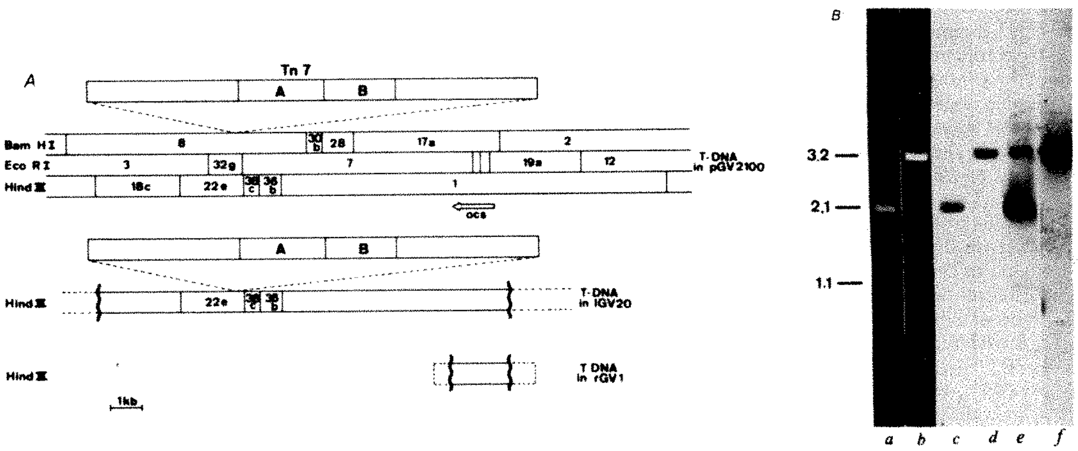

Fig. 2 Analysis of the T-DNA extent in lGV20 and in rGV1. *A*, Genomic blotting. DNA was prepared from lyophilized lGV20 tumour tissues and from rGV1 plants. Plant DNA (10 µg) was digested with *Hin*dIII and the fragments were separated by agarose gel electrophoresis, denaturated and transferred to nitrocellulose filters. Filters were hybridized against ^{32}P-labelled clones containing the right part (pGV0201)[34] and the left part (pGV0153)[34] of the pTiAch5 T-region and against pGV26[18], a ColE1 plasmid with a Tn*7* insertion. The interpretation of the resulting hybridization patterns is shown, the T-region in pGV2100 is shown for comparison. A and B are internal *Hin*dIII fragments of Tn*7* of, respectively, 2.7 and 2.25 kb[15]. The T-DNA in tumour line lGV20 is co-linear with the T-region of the inducing pGV2100. The T-DNA borders have the same approximate location as that described for most of the octopine TL-DNAs studied thus far[22–24]. No hybridization was observed using probes pGV0153 and pGV26 against rGV1-DNA. This indicated that most, if not all, of the left part of the T-DNA and the Tn*7* are absent in rGV1 DNA. Hybridization of *Hin*dIII-digested rGV1 DNA against pGV0201 revealed a single hybridization band of 3.2 kb. This band also lit up when the pTiAch5 *Bam*HI fragment 17 and *Hpa*I fragment 14, both internal T-DNA fragments situated at the right of the T-region, were used as probes. *B*, Genomic cloning. rGV1 DNA (50 µg) was digested to completion with *Hin*dIII and separated on a preparative low-gelling agarose gel. The DNA band corresponding to the 3.2-kb region was eluted from the gel[35]. Charon phage 21A was grown as described by Blattner in the detailed protocol that accompanies the Charon λ phages. Phage DNA was extracted from the purified phage as described previously[36]. The cohesive ends of the phage were annealed[36] and the phage DNA was digested with *Hin*dIII and treated with bacterial alkaline phosphatase to prevent *in vitro* packaging of the phage DNA without insert. 1 µg of insert DNA (±3.2 kb) was ligated with 10 µg Charon 21A phage DNA in 35 µl of ligase buffer[34]. Five packaging reactions, each containing 2.2 µg of recombinant DNA, were performed with *in vitro* packaging extracts prepared as described elsewhere[37]. *In vitro* packaged phages were preabsorbed to a fresh overnight BHB2600 bacterial culture[38] for 20 min at 37 °C[37], mixed with 0.7% top agar and plated at a density of 20,000 phage per 900-cm^2 plate. The plates were incubated at 37 °C for 14–16 h and refrigerated for at least 2 h before the nitrocellulose filters were applied. DNA was absorbed, denatured and bound to these filters as described previously[4]. Filters were prehybridized overnight at 42 °C in about 10 ml prehybridization mix (25 ml formamide, 2.5 ml 1 M HEPES *p*H 7.5, 2.5 ml 100 × Denhardt[39], 7.5 ml 20 × SSC[40], 1 ml 10 mg ml^{-1} yeast RNA, water to 50 ml). Denatured *Escherichia coli* DNA (50–100 µg ml^{-1}) was included in the prehybridization mix. The prehybridization and subsequent hybridization were carried out in a sealed plastic bag. The filters were hybridized with agitation at 42 °C for 24 h in prehybridization solution containing ^{32}P-labelled pGV0201 as probe. After hybridization, the filters were washed two or three times, each time with shaking, for 1 h at 50 °C in 500 ml washing solution (0.1 × SSC, 0.1% SDS), blotted dry and exposed to Fuji X-ray films with intensifying screens at −76 °C for 1–2 days. Plaques from the region of a plate corresponding to a positive signal on the autoradiogram were picked and resuspended in 0.5 ml λ phage buffer. The phage suspension was titrated and the plate containing ~100 plaques was rescreened. The process of picking positives and rescreening was repeated until ~90% of the plaques on a plate gave positive signals after screening. In this way, two independent, but identical clones were obtained. Phage DNA of these clones was prepared as described above and digested with *Hin*dIII. The 3.2-kb *Hin*dIII fragment was purified from low gelling agarose, digested with *Bam*HI and run in an agarose gel next to the *Hin*dIII fragment (lanes *a*, *b*). After transfer, the nitrocellulose filter was hybridized with purified *Bam*HI fragment 17a (lanes *c*, *d*) and subsequently with purified *Hin*dIII fragment 1 as probes (lanes *e*–*g*). The *Bam*HI fragment 17a only hybridized with the 2.1-kb fragment (lane *c*), whereas the *Hin*dIII fragment 1 hybridized with both the 2.1- and 1.1-kb fragments (lane *f*).

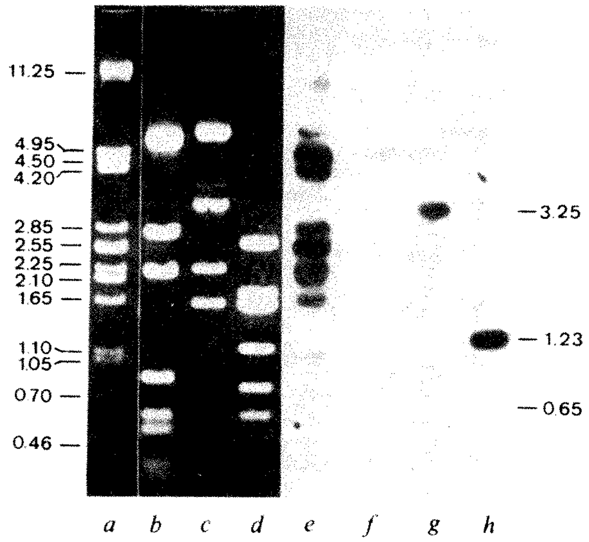

Fig. 3 T-DNA present in rGV1 mainly consists of sequences of the octopine synthase gene. Double digests, performed on different clones (lanes *b*–*d*) overlapping the whole TL-DNA, were blotted and hybridized with gcl rGV1-1 as a radioactive probe. No hybridization could be observed with the *Bam*HI–*Hin*dIII subfragments of *Bam*HI fragment 8 (lane *f*), and two out of three *Eco*RI–*Hpa*I subfragments of *Eco*RI fragment 7 (lane *g*). The two *Eco*RI–*Hpa*I subfragments which did not hybridize are the *Eco*RI–*Hpa*I fragment situated in *Bam*HI fragment 8 and the internal *Hpa*I fragment 14 of *Eco*RI fragment 7 (see Fig. 1). Hybridization of the genomic clone gcl rGV1-1 as probe against a *Bam*HI/*Sma*I double digest of *Bam*HI fragment 17a revealed that only the largest fragment hybridized (data not shown). To determine more precisely the end of the T-DNA in rGV1, a *Bam*HI/*Pvu*II double digest of *Bam*HI fragment 17a was hybridized with the genomic clone. Two of the four *Bam*HI–*Pvu*II subfragments lit up (lane *g*). This observation corresponds with the results obtained from sequence data which situated a *Pvu*II site in the 3′-untranslated region of the octopine synthase gene[32]. Lane *a*, λ digested with *Pst*I as molecular weight standard; lane *b*, *Bam*HI/*Hin*dIII double digest of clone pGV0153, containing the *Bam*HI fragment 8; lane *c*, *Eco*RI/*Hpa*I double digest of clone pGV0507[34], containing the *Eco*RI fragment 7; lane *d*, *Bam*HI/*Pvu*II double digest of clone pGV99[32], containing the *Bam*HI fragment 17a; lanes *e*, *f*, *g* and *h*, hybridization patterns obtained from, respectively, lanes *a*, *b*, *c* and *d*, using the genomic clone gcl rGV1-1 as probe.

all, of the left and middle of the T-DNA. A single hybridization band of 3.2 kb also appeared on hybridization of *Hin*dIII-digested DNA from rGV1 offspring obtained after selfing a flowering rGV1 plant, indicating that the T-DNA sequences of rGV1 passed through meiosis without rearrangements.

To confirm further and document the conclusion that the T-DNA in the rGV plants had sustained extensive deletions, genomic clones were prepared from rGV1 DNA using Charon phage 21A as vector. Two independent but identical clones were obtained (gcl rGV1-1 and gcl rGV1-2). A *Hin*dIII/*Bam*HI double digest of the gcl rGV1-1 clone gave two fragments of 2.1 and 1.1 kb, hybridizing to a probe containing the *Hin*dIII fragment 1 (Fig. 2*B*). The observed *Hin*dIII fragment of 3.2 kb presumably contained the *Bam*Hi site separating the T-region *Bam*HI fragments 17 and 2. Both the *Hin*dIII sites bordering this fragment must be derived from plant DNA sequences into which the T-DNA fragment is inserted. This conclusion was substantiated by demonstrating that (1) a probe containing *Bam*HI fragment 17a hybridized to the 2.1-kb but not to the 1.1-kb *Bam*HI–*Hin*dIII fragment (Fig. 2*B*); (2) clone gcl rGV1-1, when used as a probe against blotted TL-DNA fragments of pTiAch5, only hybridized with the right part of the TL-DNA (Fig. 3); (3) clone gcl rGV1-1, when used as a radioactive probe against a blotted *Eco*RI/*Bam*HI double digest of clone pGV105[32] (containing the *Eco*RI fragment 19a of the TL-DNA region of pTiAch5), hybridized with both the *Eco*RI–*Bam*HI fragments of the *Eco*RI fragment 19a (data not shown), indicating that the *Bam*HI site in the 3.2-kb *Hin*dIII genomic clone is indeed the site between *Bam*HI fragments 17a and 2; (4) the gcl rGV1-1 clone contains reiterated plant sequences bordering the remaining T-DNA sequences. Using either the 2.1-kb or the 1.1-kb *Hin*dIII–*Bam*HI subfragments as probes, in a Southern gel blotting hybridization against untransformed W38 tobacco DNA, a smear was obtained indicating that they both contained reiterated plant sequences.

This study proves that normal and fertile plants which still contain and express T-DNA sequences, can be regenerated from tobacco crown gall tumour cells. These 'transformed' plants are free of oncogenic properties and therefore fundamentally different from suppressed teratomata[27] or from opine-positive shoots lacking root formation and normal flower morphology[30]. The latter shoots needed to be grafted and developed into abnormal plants containing most, if not all, of the T-DNA sequences. The rGV plants described in this study, however, were shown to have lost all tumour-controlling genes by a secondary deletion in their T-DNA. The inactivation of most of the *onc* genes could therefore be essential for the regeneration of normal plants in tobacco[33].

Tumours in which TL-DNA transcripts 2, or 2 and 1, are absent seem to provide favourable conditions for the formation of shoots from nontumorous cells and from rare cells carrying a mutated T-DNA. Furthermore, our results do not distinguish whether the mutated T-DNA results from a secondary deletion after T-DNA integration, or from a mutant T-DNA present in only a few bacteria and transferred to a fraction of the transformed plant cells. If the latter were the case, we expect that Ti plasmids carrying a mutated T-DNA, similar to the one in rGV1, will efficiently transfer and integrate this T-DNA without inducing tumorous growth. The use of these mutated Ti plasmids as suitable vectors for gene transfer into dicotyledonous plants will then require: (1) appropriate conditions to regenerate plants from undifferentiated callus cultures; (2) a selective method to recover 'transformed' cells; indeed, it is likely that T-DNA-positive cells will be present only as a minority amongst wild-type cells.

It is important to stress that genes, such as opine synthases, introduced into plants via modified Ti plasmids, are stably maintained not only in somatic cells, but also through meiosis. Furthermore, independent insertions were shown to take place at different loci.

Finally, the finding that T-DNA-containing plants of the rGV1 type are fully susceptible to Ti plasmid-mediated transformation opens up the possibility of successive insertions of different genes in the same plant.

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Exon shuffling: mapping polymorphic determinants on hybrid mouse transplantation antigens

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The mouse major transplantation antigens H-2K, H-2D and H-2L are highly polymorphic cell-surface glycoproteins which may serve as recognition elements in cell–cell interactions[1]. Each antigen possesses a number of alloantigenic determinants defined by antisera of various specificities. Recently, monoclonal antibodies have been produced which redefine and extend our knowledge of these determinants[2,3], but structural information has not yet been correlated with the serological definition of the antigens. We have previously reported the molecular cloning of genes for H-2L^{d} and H-2D^{d} transplantation antigens from the BALB/c mouse and the expression of these genes in mouse L cells[4,5]. To localize the serological determinants to discrete regions of the H-2 protein, we have now constructed new H-2 antigen genes by joining together fragments of the *H-2L*^{d} and *H-2D*^{d} genes. In L cells, these genes direct the synthesis of hybrid H-2 proteins and by using monoclonal antibodies of defined specificities, we have mapped classically defined serological specificities to structurally defined domains of the transplantation antigen protein. We conclude that polymorphic determinants recognized by monoclonal antibodies are located in functionally distinct portions of the protein.

We obtained genomic clones containing *H-2L*^{d} and *H-2D*^{d} genes from a BALB/c mouse genomic library[4,5]. The DNA sequence of the entire 4.5-kilobase (kb) *H-2L*^{d} gene has been determined (refs 4, 6 and G.A.E., unpublished data). The three exons at the 5′ end of the *H-2D*^{d} gene have been sequenced and the derived amino acid sequence agrees precisely with the known H-2D^{d} protein sequence[5]. These genes were introduced into thymidine kinase(tk)-deficient mouse L cells by DNA-mediated gene transfer[7,8] and two cloned L cell lines were obtained which expressed H-2L^{d} and H-2D^{d} determinants in the presence of the parental *H-2*^{k} background. Thus, the H-2 phenotypes of these cell lines are H-2K^{k}, D^{k}, D^{d} (cell line T4.8.3) and H-2K^{k}, D^{k}, L^{d} (cell line T1.1.1). Using a panel of 13 monoclonal antibodies having H-2 specificities, we screened these cell lines for reactivity (Table 1). All three H-2L^{d}-specific monoclonal antibodies reacted with T1.1.1 cells but not with T4.8.3 cells. However, those antibodies reacting with antigens encoded by the *D* region of the *H-2*^{d} haplotype could not distinguish between determinants shared between H-2L^{d} and H-2D^{d} and those unique to H-2D^{d}, because of a lack of appropriate recombinant strains[9]. The availability of transformed cell lines separately expressing H-2D^{d} and H-2L^{d} antigens has allowed us to assess the precise specificities of the antibodies. Six monoclonal antibodies were found to be specific for the H-2D^{d}-producing cell line. The remaining three antibodies showed significant reactivity with both H-2L^{d} and H-2D^{d}, indicating specificity for a shared determinant (Table 1).

In order to more precisely define the structural basis of the allodeterminants recognized by these antibodies as well as allorecognition by T lymphocytes, we constructed hybrid *H-2* genes by combining exons from the cloned *H-2L*^{d} and *H-2D*^{d} genes. Several restriction endonuclease cleavage sites, including two *Xba*I sites, were conserved between the two sequences. After subcloning the respective genes into plasmid vectors, *Xba*I fragments containing the 5′ coding region of the genes were excised, exchanged and re-ligated with the 3′ end of the genes using T$_4$ DNA ligase (Fig. 1). This reconstruction resulted in two hybrid genes. The gene carried on the pG18 plasmid contains the three 5′ exons (L, N and C1) of *H-2L*^{d} and the 3′ exons of the *H-2D*^{d} gene. pG24 contains the first three exons of *H-2D*^{d} and the 3′ end of *H-2L*^{d} (Fig. 1*b*). If correctly expressed, the products of these genes would contain the N and C1 external domains of one transplantation antigen molecule and the C2 domain of the other.

To determine whether these hybrid genes could be expressed in mouse fibroblasts, pG18 and pG24 DNA was introduced into L(tk^{-})cells by DNA-mediated gene transfer[4,5]. Cloned cell lines from the resultant transformants were screened with a panel of monoclonal antibodies specific for H-2D^{d} and H-2L^{d} determinants. IgG class monoclonal antibodies were tested for binding by radioimmunoassay (Table 1*a*). One of the anti-H-2L^{d} antibodies, 28.14.8, reacted specifically with pG24-containing cells (T37.2.1) but not with pG18-containing cells, indicating that the determinant recognized by this antibody depends on the presence of the C2 domain of the H-2L^{d} molecule for expression. Although conformational interactions of widely separated portions of the molecule could be responsible for unmasking the monoclonal antibody determinant, the most likely explanation is that the 28.14.8 antibody recognizes a determinant physically located on the C2 domain of the molecule. Similarly, the determinants recognized by antibodies 34.5.8, 28.8.6 and 34.1.2 map to the N or C1 domains of the H-2D^{d} protein, the specificity of antibody 34.2.12 maps to the C2 domain of H-2D^{d}, and the specificity of antibody 30.5.7 maps to the N or C1 domains of H-2L^{d}. IgM class monoclonal antibodies, screened by flow microfluorimetry (Table 1*b*) show that H-2D^{d} specificities 34.4.21 and 23.5.21 map to the N or C1 regions of H-2D^{d} and the 23.10.1 determinant maps to the N or C1 domains of the H-2L^{d} molecule (Table 1*b*). Monoclonal antibodies 34.4.20, 27.11.13 and 28.11.5, which show cross-reacting specificities, react in all cases with both hybrid gene products. However, significant quantitative differences in binding suggest that these determinants are not identical. For example, antibody 34.4.20 binds significantly better to H-2D^{d}

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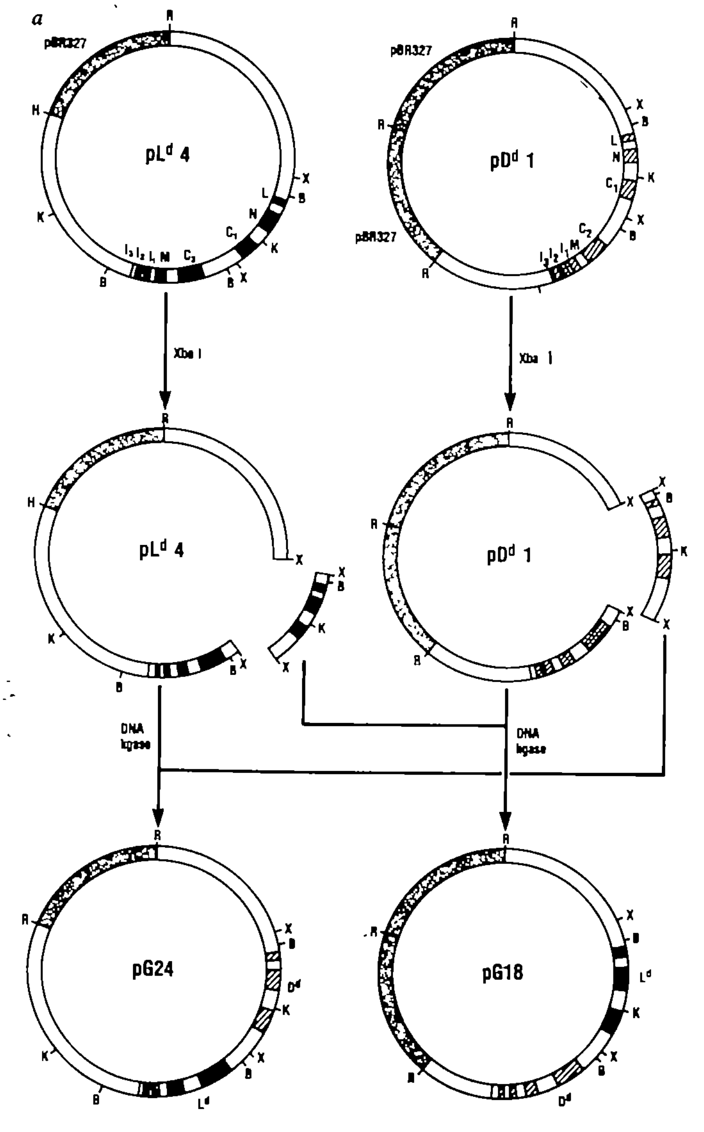

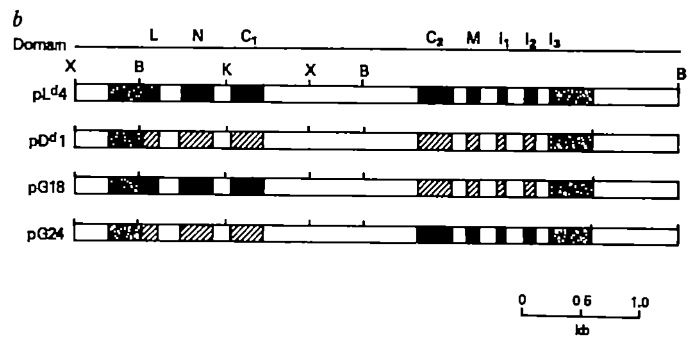

Fig. 1 Construction of recombinant genes from the cloned *H-2D*^{d} and *H-2L*^{d} genes. *a*, Fragments of the bacteriophage DNA containing *H-2* genes were subcloned into plasmid vectors for gene manipulation experiments. A 12-kb *Eco*RI-*Hin*dIII fragment from Ch4AH-2L^d (ref. 4) was subcloned into the *Eco*RI and *Hin*dIII sites of pBR327 (ref. 19), producing the plasmid pL^{d}4. Similarly, a subclone containing the *H-2D*^{d} gene, pD^{d}1, was prepared by subcloning the 8-kb *Eco*RI fragment of Ch4AH-2D^d (ref. 5) into the *Eco*RI site of pBR327. (As an artefact of this cloning procedure, pD^{d}1 contains two copies of pBR327 joined at the *Eco*RI sites in the same orientation.) Recombinants between the *H-2L*^{d} and *H-2D*^{d} genes were constructed by cleaving both plasmids with *Xba*I. The resultant fragments were purified by electrophoresis on 1% low melting point agarose gels and elution at 65 °C, followed by purification on DE-23 columns. The 1.6-kb *Xba*I fragment from pL^{d}4 was inserted into the 15-kb *Xba*I fragment of pD^{d}1 using 1 unit of T_4 DNA ligase in 10 mM Tris-HCl *p*H 7.6, 1 mM dATP, 0.5 mM EDTA, 6 mM $MgCl_2$ and 50 mM NaCl for 6 h at 14 °C. The 15-kb fragment was treated with bacterial alkaline phosphatase before ligation to prevent recircularization. The ligated DNA was reintroduced into *E. coli* LE392 by $CaCl_2$-induced transfection[17]. Colonies were screened for the insert having the correct orientation by 'mini-DNA' preparations[18] and the resulting hybrid plasmid, pG18, extensively mapped by restriction endonuclease cleavage to confirm the reconstruction. Similarly, pG24 was constructed by ligating the 1.6-kb *Xba*I fragment from pD^{d}1 to the 15-kb fragment from pL^{d}4. *b*, The resulting hybrid genes were recombined at the *Xba*I site located within the 1.3-kb intervening sequence separating the exons coding for the C1 and C2 domains. The hybrid *H-2* gene carried on pG18 contains the first three exons, corresponding to the L, N and C1 domains, of *H-2L*^{d} and the following five exons of *H-2D*^{d}. The gene on pG24 contains the L, N and C1 coding regions of *H-2D*^{d} and the 3′ domains of *H-2L*^{d}. Cross-hatched areas represent the regions coding for *H-2L*^{d}; solid regions represent those coding for *H-2D*^{d} domains. Stippled areas represent pBR327 (*a*) or the 5′ and 3′ untranslated regions of the *H-2* mRNA (*b*). L, N, C1, C2, M, I1, I2 and I3 refer to the regions of the protein encoded by each exon labelled by analogy to the *H-2K*^{b} prototype structure[10]. X, *Xba*I; B, *Bam*HI; H, *Hin*dIII; K, *Kpn*I; R, *Eco*RI cleavage sites.

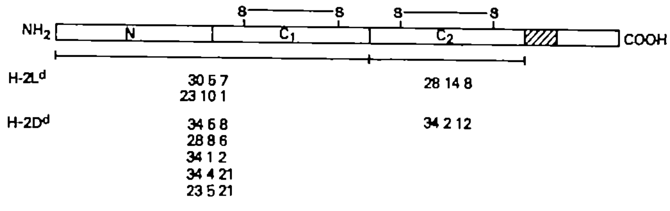

Fig. 2 Localization of polymorphic determinants on the external domains of H-2 proteins. This diagram summarizes the reactivity of H-2L^d- and H-2D^d-specific monoclonal antibodies with hybrid *H-2* genes. The reacting monoclonal antibody is listed in relation to the globular subunit of the protein to which it binds. The determinants recognized by monoclonal antibodies 30.5.7 and 28.14.8 have previously been shown to correlate with the classical serological specificities H-2.65 and H-2.64, respectively[2,12,13].

and the pG24 gene product (N and C1 of H-2D^d; C2 of H-2L^d) than to H-2L^d or the pG18 gene product. Monoclonal antibodies specific for N–C1 or C2 domains have been used to immunoprecipitate molecules from both T37.1.3 and T37.2.1 (G.A.E., unpublished). These immunoprecipitated proteins have molecular weights of 45,000 daltons and associate with β_2-microglobulin, consistent with the predicted hybrid protein structures.

This study demonstrates the feasibility of *in vitro* genetic recombination to generate hybrid, and thus allogeneically novel transplantation antigens which are expressed in a normal fashion on the cell surface. Thirteen determinants defined by monoclonal antibodies were active in the hybrid gene products. No determinants present in the two parental gene products were lost in either of the two hybrid gene products. Therefore the domain structure of the transplantation antigens may also define a functional separation of polymorphic antigenic sites. The location of antigenic determinants can be correlated with the domain structure of the molecule. Polymorphic determinants recognized by monoclonal antibodies are located on the N, C1 and C2 domains. Although the C2 domain shows significantly less variability in amino acid sequence than the N and C1 regions[10,11], polymorphic alloantigenic determinants may be located in this region of the protein also. Interestingly, monoclonal antibody 28.14.8 was originally found to be cross-reactive with the H-2D^b antigen. This study maps this determinant to the C2 domain and recent amino acid sequence data demonstrate that the C2 domains of H-2L^d and H-2D^b are identical[11]. In addition, monoclonal antibodies 28.14.8 and 30.5.7 correlate with serological specificities H-2.64 and H-2.65, respectively, originally defined by classical serology[2,12,13], which map to structurally distinct portions of the H-2L^d molecule (Fig. 2). Thus, the spatial relationships of polymorphic

Table 1 Monoclonal antibody binding to hybrid H–2 gene products

a

	Antibody reactivity*								
Monoclonal antibody	16.1 2	34 4.20	27 11 13	30.5 7	28 14.8	34 5 8	28.8.6	34.1.2	34.2.12
Specificity	K^kD^k	D^dL^d	D^dL^d	L^d	L^d	D^d	D^d	D^d	D^d
Cell (gene)									
DAP-3	3,049	120	119	110	115	125	118	124	150
T1.1.1 (L^d)	2,024	1,816	1,212	2,925	2,335	107	95	157	116
T4.8.3 (D^d)	2,534	4,748	2,119	88	140	4,292	3,820	5,022	3,713
T37.1.3 (L^dD^d)	2,536	1,850	1,703	4,219	161	169	184	211	3,799
T37.2.1 (D^dL^d)	2,016	4,262	3,016	113	4,193	4,319	3,706	3,933	171

b

	Antibody reactivity*			
Monoclonal antibody	34 4.21	23.5.21	23.10 1	28.11.5
Specificity	D^d	D^d	L^d	D^dL^d
Cell (gene)				
DAP-3	28.0	25.8	24.5	28 1
T1.1.1 (L^d)	36.5	23.4	284.2	202.5
T4.8.3 (D^d)	297.0	242.8	26.5	335.3
T37.1.3 (L^dD^d)	31.4	23.5	238.1	73.2
T37.2.1 (D^dL^d)	856.2	338.3	19.2	342.5

Cloned *H–2* genes were introduced into thymidine kinase-deficient mouse L cells (DAP-3) by DNA-mediated gene transfer using the herpes virus thymidine kinase gene[16] as a selectable marker. The cloned cell lines T1.1.1, containing an *H–2L^d* gene[4] and T4.8.3, containing an *H–2D^d* gene[5], have been described elsewhere. These cloned cell lines have been maintained in continuous culture for over 6 months without loss or alteration of H–2 expression. Monoclonal antibodies specific for H–2D^d and/or H–2L^d determinants have been characterized previously[2,9]. *a*, IgG class monoclonal antibodies having specificities for H–2L^d or H–2D^d were screened for binding to transformed L cells containing *H–2L^d* *H–2D^d* or one of the two hybrid genes pG18 or pG24. Screening was performed by radioimmunoassay in Falcon flat-bottom 96-well microtitre plates. 10^6 cells were added to each well and incubated overnight at 37 °C in Dulbecco's modified Eagle's medium supplemented with glutamine, hypoxanthine, ammopterine, thymidine and 10% fetal calf serum[4]. After washing, 50 µl of a 1:1 dilution of hybridoma supernatant were added and the cells were incubated for 60 min at 4 °C. The cells were extensively washed, ^{125}I-labelled goat anti-mouse IgG added to each well, and the cells incubated for an additional 60 min at 4 °C. After extensive washing, cells were lysed with 1% Triton X-100, supernatants collected with a Titertek supernatant collection system, and radioactivity determined in a γ-spectrometer. Results are expressed as c.p.m. ^{125}I bound above background Each value represents the mean of triplicate samples with a standard deviation of less than 3% of the mean. Monoclonal antibody 28.8.6 was previously reported to react only with H–2K^b and H–2D^b (ref. 3) Additional cross-reactivities with H–2D^d have been found by radioimmunoassay and flow microfluorimetry (K.O , unpublished) *b*, IgM class monoclonal antibody binding was determined by flow microfluorimetry as described[4,5] using fluorescein-conjugated goat F(ab′)$_2$ anti-mouse F(ab′)$_2$. The results represent mean fluorescence normalized to gain 1 The fluorescent profile showed a single population of stained cells. Standard deviation of the mean fluorescence was less than 60 fluorescence units.

* Only the reactivity relevant to this study is shown.

determinants previously assessed only by competitive binding of monoclonal antibodies[2] may, using these techniques, be correlated with distinct molecular structures.

Monoclonal antibodies against H–2 specificities have been shown to specifically block cell-mediated lympholysis by T cells activated against H–2 allodeterminants[14,15]. Specifically, monoclonal antibody 28.14.8 has been shown to block both allogeneic and H–2-restricted cell-mediated lympholysis[14]. As shown here, the determinant recognized by antibody 28.14.8 is located on the C2 domain, a region which may, therefore, sterically affect H–2 antigen recognition by T lymphocytes. The use of H–2 antigens modified by genetic engineering coupled with *in vitro* mutagenesis makes feasible the complete structural and functional definition of allodeterminants on transplantation antigens and their recognition by T cells.

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Amplification of immunoglobulin λ constant genes in populations of wild mice

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The λ immunoglobulin light chain (Igλ) locus of BALB/c inbred mice consists of two variable region gene segments (V_λ)[1–3], and four constant region gene segments (C_λ)[1,2,4,5]. Each C_λ gene segment is associated with a unique joining segment (J_λ)[2,4–7], and they are organized in two paired units, J_3C_3–J_1C_1 and J_2C_2–J_4C_4 (refs 4, 8). Using cDNA probes specific for C_{λ_1} and C_{λ_2} (ref. 9) we have analysed the genomic organization of the C_λ gene segments in wild-derived and inbred strains of mice. Although Southern blots of the genomic DNA of inbred mice show a constant pattern of hybridization, wild-derived mice show a high degree of variation in the number, size and intensity of hybridizing fragments. We have now found that, per haploid genome, mice of a *Mus musculus musculus* stock isolated from Sladeckovce, Czechoslovakia (CzII) have at least 12 C_λ segments, and mice of a *Mus musculus domesticus* stock 'Centreville Lights' from Centreville, Maryland (CL) have at least 8 C_λ segments. There appears to have been relatively recent amplifications of the C_λ gene segments in wild mice.

The immunoglobulin C_λ J_3C_3–J_1C_1 and J_2C_2–J_4C_4 paired units share a similar internal organization[4,5,8] (Fig. 1). Considerable sequence homology exists between C_{λ_2} and C_{λ_3}; somewhat

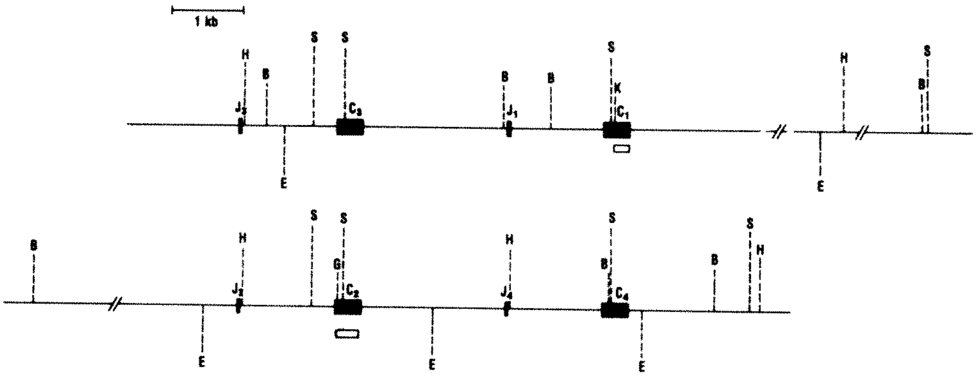

Fig. 1 Partial genomic map of inbred mouse C_λ locus constructed from data of Selsing *et al.*[8]. Black boxes denote coding sequences and open boxes beneath indicate regions included in cDNA hybridization probes. Restriction endonuclease sites are indicated and labelled thus: B, *Bam*HI; E, *Eco*RI; G, *Bgl*I; H, *Hin*dIII; K, *Kpn*I; S, *Sac*I. We prepared λ_1 cDNA from myeloma MOPC104E mRNA as described[10] with the following modifications: no actinomycin D was present in the cDNA synthesis reaction; whole DNA polymerase I was used in the second strand synthesis; *E. coli* strain MC1061[11] was transformed by the recombinant pBR322. Colony hybridization[12] using a cloned cDNA derived from the λ_1-secreting plasmacytoma, HOPCl (from S. Cory) was used to identify clones containing M104E λ_1 cDNA insert. A C_{λ_1}-specific cDNA fragment was generated by double-digestion of the MOPC104E λ_1 recombinant plasmid DNA with *Kpn*I and *Eco*RI, followed by purification by polyacrylamide gel electrophoresis[13]. A recombinant plasmid, pλII-1, containing M315 λ_2 cDNA was provided by A. L. M. Bothwell[9]. *Bgl*I digestion of pλII-1 and polyacrylamide gel purification generated a C_{λ_2}-specific cDNA fragment. The C_{λ_1}- and C_{λ_2}-specific cDNA fragments were identified on the basis of restriction enzyme mapping and comparison of results of hybridization to inbred mouse genomic DNA with those of Blombert *et al.*[4].

less homology is shared by C_{λ_1} and C_{λ_4} (ref. 8). Hybridization of C_{λ_1} cDNA to *Eco*RI digested inbred mouse liver genomic DNA identifies a 10 kilobase (kb) fragment containing the C_{λ_1} gene segment, and a fainter 2.8 kb fragment containing the C_{λ_4} gene segment (Fig. 2*a*, lane 1). There is no hybridization to C_{λ_2} or C_{λ_3} (ref. 4). Similarly, C_{λ_2} cDNA hybridizes to 10-kb and 3.2-kb fragments containing the C_{λ_3} and C_{λ_2} gene segments, respectively, but not to C_{λ_1} or C_{λ_4} (Fig. 2*b*). These *Eco*RI band patterns are representative of those observed for all inbred strains examined thus far: AKR/N, BALB/cAnπ, BALB/cJ, C57BL/Ka, C58, C3H/HeN, SJL/J, SJA, SWR/J, DBA/2, PL/J, BSVS, RF/J, 129/J, ST/bJ, SM/J, RIII, AuSSJ, CBA/ICR and CBA/T6T6. In striking contrast to the patterns observed with inbred strains are those obtained in many wild mouse populations, represented in Fig. 2 by two examples, CL (lane 2) and CzII (lane 3). The CL mouse *Eco*RI patterns contain six fragments that strongly hybridize to the C_{λ_1} probe and six that hybridize to the C_{λ_2} probe, while the same probes hybridize to eight and seven fragments, respectively, of CzII DNA digested with *Eco*RI.

Several explanations might account for the extra bands seen in CL and CzII DNA. Incomplete digestion by the endonuclease could give rise to more fragments hybridized to the cDNA probes. The same patterns for both inbred and wild-derived mice, however, have been seen repeatedly in stringently controlled digestion, blotting and hybridization conditions. Moreover, incomplete digestion of the DNA surrounding the C_{λ_3}–C_{λ_1} unit should give rise to fragments larger than the 10 kb fragments seen in inbred mice. Instead, all but one of the

Table 1 Number of fragments strongly hybridizing to C_{λ_1} and C_{λ_2} probes

Genomic DNA source	cDNA probe	*Eco*RI	*Bam*HI	*Hin*dIII	*Sac*I
BALB/c	C_{λ_1}	2	1*	2	3
(Inbred)	C_{λ_2}	2	2	2	1
M. musculus domesticus	C_{λ_1}	6	4	5	6
(Centreville)	C_{λ_2}	7	5	5	5
M. musculus musculus	C_{λ_1}	8	6	6	8
(Sladeckovce)	C_{λ_2}	7	9	6	6

Number of DNA fragments that strongly hybridize to C_{λ_1} and C_{λ_2} probes. The number of strongly hybridizing fragments generated under conditions as described previously is indicated. Highly intense bands, which may indicate the presence of multiple copies of a gene fragment, are counted as one band.

* Only one of the two *Bam*HI fragments hybridizes strongly. The C_{λ_4} gene fragment hybridizes very weakly and was therefore not counted.

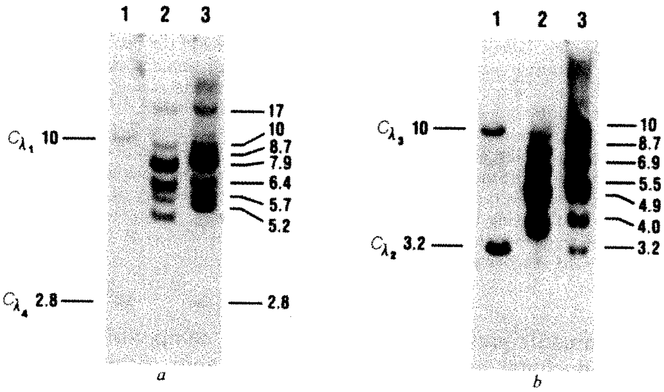

Fig. 2 *Eco*RI digested BALB/c, CL and CzII genomic DNA hybridized to C_{λ_1}- and C_{λ_2}-specific cDNA probes. Genomic DNA was prepared from mouse liver by homogenization in 5% citric acid, isolation of nuclei, lysis at 65 °C in 50 mM Tris-HCl *p*H 9.5, 100 mM EDTA, 1% SDS, sequential treatment with 500 $\mu g\ ml^{-1}$ pronase and 10 $\mu g\ ml^{-1}$ RNase, phenol extraction and ethanol preciptation. The data shown were generated by digestion of 10 µg of each DNA sample with restriction endonuclease (3–5 U per µg DNA) for 2–12 h at 37 °C, electrophoresis on 0.7% agarose followed by blotting onto nitrocellulose[14]. Before hybridization, blots were soaked in 6 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M Na citrate), 10 × Denhardt's solution[15], 0.1% SDS and 60 $\mu g\ ml^{-1}$ sheared salmon sperm DNA for 2–4 h at 65 °C. 1×10^7 c.p.m. of C_{λ_1} or C_{λ_2} cDNA labelled by nick-translation[16] with [α-^{32}P]dCTP to $\sim 2 \times 10^8$ c.p.m. per µg DNA was hybridized to the genomic DNA blot overnight at 65 °C, in the same conditions as for prehybridization. The hybridized blots were washed once in 10 × Denhardt's solution, 2 × SSC, 0.1% SDS, 1 mM EDTA for 30 min at 65 °C, twice in 2 × SSC, 0.1% SDS, 1 mM EDTA for 30 min at 65 °C and twice in 0.2 × SSC, 0.1% SDS, 1 mM EDTA for 30 min at 65 °C. *a*, BALB/c (1), CL (2) and CzII (3) liver DNA hybridized to C_{λ_1} cDNA. *b*, Hybridization of the same DNAs to C_{λ_2} cDNA. The sizes (kb) of the BALB/c and CzII bands are indicated. Sizes were determined by comparison of the mobility of the hybridized fragment to a standard curve generated from linear regression analysis of 35 gels where *Hin*dIII fragments of phage λ DNA were used as DNA size markers. BALB/c fragments containing C_{λ_1}, C_{λ_2}, C_{λ_3} and C_{λ_4} genes were identified by comparison to published photographs[4] and restriction enzyme maps[8]. To ascertain that the hybridizations were indeed to C_λ coding regions and not to sequences related to the 3′ untranslated region, the λ_1 cDNA probe was fractionated to generate a fragment that contained coding region only and no 3′ untranslated sequence. When this fragment was labelled and hybridized to *Eco*RI digested BALB/c, CL and CzII genomic DNA under conditions as described above, we observed the same number of C_λ bands as seen in *a*, thus confirming hybridization to coding sequence.

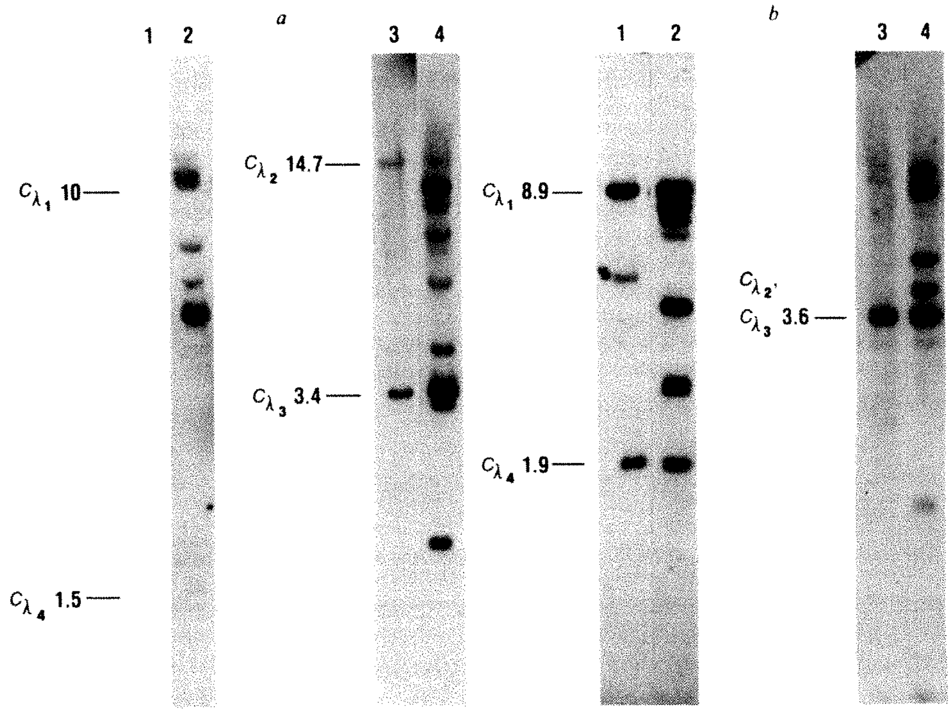

Fig. 3 Restriction endonuclease digestion (*a*, *Bam*HI and *b*, *Sac*I) of BALB/c (lanes 1, 3) and CzII (lanes 2, 4) DNA hybridized to C_{λ_1} cDNA (lanes 1, 2) and C_{λ_2} cDNA (lanes 3, 4). Digestion, hybridization and wash conditions were as described in Fig. 2 legend. Sizes of inbred mouse DNA fragments are indicated.

additional bands are smaller than 10 kb. Similarly, incomplete digestion products of the C_{λ_2}–C_{λ_4} unit would be larger than the 2.8 kb fragment of inbred mouse DNA hybridizing to the C_{λ_1} cDNA probe. Although the additional bands in CL and CzII are larger than 2.8 kb, the intensity of those bands is so much greater than that of the inbred mouse 2.8 kb fragment that it seems unlikely they are the result of incomplete digestion of that unit. As further evidence of completeness of digestion, when the same filters are stripped of labelled C_λ probe and rehybridized to probes independent of C_λ (for example, V_λ, C.L.S., manuscript in preparation), multiple bands are not present in CL and CzII DNA. Therefore, the patterns seen with the C_λ probes may be explained by the occurrence of multiple C_λ sequences in the DNA of CL and CzII.

Mutations generating *Eco*RI sites in the coding region of any of the inbred mouse C_λ gene segments would also give rise to additional fragments. However, the size range and number of the CL and CzII fragments is inconsistent with such an interpretation. In the unlikely event that multiple *Eco*RI sites have arisen in the coding region, which is only 318 base pairs (bp) in length, the fragments resulting from *Eco*RI digestion would, with the possible exception of those immediately adjacent to flanking regions, be very small and difficult to detect by Southern hybridization. A third basis for multiple bands in wild mice might be polymorphism and heterozygosity. Polymorphism does appear to exist between CL and other mice derived from the same population in Centreville, Maryland (C.L.S. and M.P., unpublished data). The difference between individuals, however, is not seen as multiple bands in one case and few in the other, but involves a difference of only one or two bands, depending on the restriction enzyme used. Heterozygosity may exist, because these colonies are maintained in an outcrossed fashion. However, all CzII individuals so far examined have the same pattern as do $(\text{BALB/c}\times\text{CzII})_{F_1}$ animals, which show BALB/c bands plus all CzII bands. Further, pooled genomic DNAs of mice derived from various localities in Eastern Europe and South-East Asia show a complex hybridization pattern similar to that of CzII. Even if polymorphism exists, it does not explain the large increase in number of C_λ bands seen in some of the wild populations. Instead, the patterns of C_λ fragments strongly suggest that more C_λ genes are present in these mice than in inbred strains.

To substantiate the multiple gene copy interpretation, we digested the same DNAs with *Bam*HI, *Hin*dIII, *Kpn*I and *Sac*I and hybridized with C_{λ_1} and C_{λ_2} probes. Multiple intense bands were visible in the wild mouse DNAs for all enzymes used, while inbred mice consistently had few bands (Table 1). Figure 3 shows examples of hybridizations to inbred (lanes 1, 3) and CzII (lanes 2, 4) DNA digested with *Bam*HI (Fig. 3*a*) and *Sac*I (Fig. 3*b*). The hybridization intensity of some of the wild mouse DNA fragments is substantially greater than that of the fragment containing the inbred mouse gene homologous to the cDNA probe. For example, the 7.9 kb *Eco*RI band of CzII is much darker than the 10 kb band of BALB/c, which contains the C_{λ_1} gene (Fig. 2*a*). Similar highly intense bands are visible in *Bam*HI digested CzII DNA hybridized to either the C_{λ_1} or the C_{λ_2} cDNA probe. The inbred mouse *Bam*HI fragments containing the C_{λ_1} and C_{λ_2} genes do not hybridize as strongly. This suggests that more than one copy of C_λ gene sequence is present in the strongest hybridizing fragments. On the basis of the intensity and number of additional fragments generated by digestion with five different restriction endonucleases, we conclude that CL and CzII mice have more C_λ genes than do inbred mice. In light of the fact that the inbred mouse C_{λ_1} and C_{λ_4} sequences do not cross-hybridize with the C_{λ_2} and C_{λ_3} sequences, it is apparent that the CL and CzII mice have multiplied sets of both C_{λ_1}-analogous and C_{λ_2}-analogous genes. Exact determination of the number of C_λ genes in the wild mice is not yet possible, but the independent C_{λ_1} and C_{λ_2} cDNA hybridization results indicate the presence of at least 8 in CL mice and 12 in CzII mice.

The four C_λ genes of inbred mice are presumed to be the product of duplication of a C_{λ_x}–C_{λ_y} unit[4,6,8], with C_{λ_x} as ancestor of C_{λ_2} and C_{λ_3}, and C_{λ_y} of C_{λ_1} and C_{λ_4}. Possible mechanisms of amplification include duplication of single C_{λ_x} or C_{λ_y} genes, sequential duplication of a C_{λ_x}–C_{λ_y} unit, or simultaneous duplication of multiples of the unit. When both the number and intensity of C_λ bands in CL and CzII are considered, the increase in number of C_{λ_x} and C_{λ_y} genes appears concordant, which more strongly supports a unit mechanism of duplication than the single gene duplication mechanism. Although it is not yet clear whether analogues to both inbred mouse C_λ units are involved, it seems that an analogue to the C_{λ_3}–C_{λ_1} unit has participated in the duplication process, as indicated by the intensity of bands hybridizing to C_{λ_1} cDNA. In duplication of a unit containing a C_{λ_y} gene that strongly hybridizes to the C_{λ_1} cDNA probe, there would be concordant duplication of a C_{λ_x} gene that strongly hybridizes to the C_{λ_2} cDNA probe. The hybridization patterns of CL and CzII are consistent with such a prediction.

The additional C_λ genes described in CL and CzII may be ancestral genes that segregated away from the progenitors of inbred strains, or they may represent a recent amplification of C_λ in some wild mouse populations. If the CL and CzII mice carry ancestral conserved genes, evolutionary drift of the DNA sequence would probably generate weaker hybridization bands than are observed. Thus, the strength of hybridization of inbred mouse C_{λ_1} and C_{λ_2} cDNA probes to CL and CzII C_λ genes seems to support recent amplification. There is evidence that duplication of C_λ genes has already occurred fairly recently in the mice from which inbred strains are derived, as indicated by the homology of the two C_λ units[4,6,8].

It is generally accepted that a single C_κ gene is adequate for expression of a large repertoire of immunoglobulin κ light chains in mice, so the role of multiple C_λ genes is not obvious. If J_λ amplification occurs concurrent with C_λ amplification, immunoglobulin λ diversity might ultimately be influenced. It may be that amplification of C_λ genes is associated with amplification of V_λ genes as well, but as the location of mouse V_λ genes relative to C_λ genes remains unknown, this possibility cannot yet be assessed.

We are currently preparing genomic clones from CzII mice to elucidate further the nature of the C_λ amplification at the DNA level. In addition, experiments at the RNA and protein levels are in progress to ascertain whether the additional C_λ genes are expressed.

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Inherited deletion of immunoglobulin heavy chain constant region genes in normal human individuals

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The existence of specific probes for human genes makes it feasible to study genetic abnormalities, both inherited and acquired, at the level of the genome. In this respect, the antibody genes of man are of particular interest as they represent a multigene family expressed in many leukaemias and immunodeficiency diseases. Furthermore, selective deficiency of immunoglobulins has been described in healthy individuals[1]. Normally, human adults express five types of immunoglobulin—IgM, IgD, IgG, IgE and IgA (defined by the class of heavy chain constant region). Subclasses are also known in IgG (IgG1, IgG2, IgG3 and IgG4) and IgA (IgA1 and IgA2) in which the immunoglobulins contain $\gamma 1$, $\gamma 2$, $\gamma 3$ or $\gamma 4$ and $\alpha 1$ or $\alpha 2$ C_H regions, respectively. Recently, a healthy Tunisian person was described who showed abnormal patterns of immunoglobulin expression[2]. The serum immunoglobulin of this individual, designated TAK3, was confined to IgM, IgD, IgG3, IgE and IgA2. We have now used cloned C_H-gene probes to study the DNA of TAK3 as well as two brothers, also Tunisian but apparently unrelated to the individual TAK3, and who show a similar immunoglobulin abnormality. We found that in these cases there seems to have been a large chromosomal deletion which includes three γ genes, an α gene and a pseudo-ε gene. This deletion accounts for the simultaneous absence of certain H-chain subclasses. These results illustrate that the human immunoglobulin gene locus is capable of undergoing rapid change, which is particularly apparent within small populations in which consanguinity is common.

The patterns of hybridization with C_H-gene probes of the DNA from TAK3, and the brothers TOU and EZZ were studied by Southern filter hybridization, and compared with DNA from individuals with normal immunoglobulin expression (both of Tunisian and European origins) plus a person, TAK14 (the daughter of TAK3), who is heterozygous for the immunoglobulin abnormality. The state of the γ genes in TAK3 and TOU was examined using a $\gamma 3$ probe (clone p3.6RH4.2, which contains a 3.6-kilobase (kb) *Eco*RI–*Hin*dIII fragment encompassing the whole $\gamma 3$ gene coding region[3]) and a $\gamma 4$ probe (clone pBRH4.1, which contains an analogous fragment encompassing the $\gamma 4$ gene[3]). These probes cross-hybridize with the genes for the various γ subclasses. A high degree of restriction fragment polymorphism has been observed in the γ genes; both the $\gamma 4$ probe (Fig. 1*a*) and the $\gamma 3$ probe (Fig. 1*b*) detect between five and seven hybridizing *Bam*HI fragments as exemplified by genomic DNAs from different individuals including a placental DNA obtained in Cambridge (Fig. 1*b*, lane 4) and two other Tunisian DNAs, designated LAT and BOU (Fig. 1*a*, lanes 1, 4). Common 9-, 11- and 12-kb fragments were present in all these DNAs, and in one DNA an additional 23-kb band was detected (Fig. 1*a*, lane 4) which we also observed in other Tunisian DNAs (data not shown).

When we analysed hybridization of the $\gamma 4$ probe with *Bam*HI-digested DNA from TAK3, we observed only two bands (8 and 10.5 kb; Fig. 1*a*, lane 3) whereas hybridization with the $\gamma 3$ probe showed only the 10.5-kb fragment (Fig. 1*b*, lane 2). Presumably, this 10.5-kb *Bam*HI fragment detected in TAK3 DNA represents the $\gamma 3$ gene (because only the IgG3 subclass is expressed in TAK3). The unusual size of this fragment may result from an altered restriction site which occurred during the deletion event. The origin of the second, more weakly hybridizing component could be the pseudo-γ gene[3,4] or even a partially deleted γ gene. Neither of the bands observed in TAK3 corresponds with the fragments detected in any of the other DNAs except TAK14 DNA (Fig. 1*a*, lane 2; *b*, lane 3) which is heterozygous for the immunoglobulin defect found in TAK3. The heterozygosity of TAK14 is demonstrated by the presence of a normal pattern of five bands plus the two unusual bands detected in TAK3 DNA. These data suggest that the simultaneous absence of $\gamma 1$, $\gamma 2$ and $\gamma 4$ subclasses in the homozygous individual TAK3 is due to an extensive deletion of the region of chromosome 14 which carries the γ genes[5–7]. The hybridization of the $\gamma 3$ probe to *Bam*HI-digested TOU DNA is also shown in Fig. 1*b* (lane 1). Interestingly, a similar γ deletion has occurred in TOU to that which occurred in TAK3, except that we could only detect the 10.5-kb hybridizing band in TOU DNA. These results with γ-gene probes show that two individuals displaying abnormal patterns of γ-globulin expression seem to do so because of inherited deletions of the respective γ genes.

A further investigation of the C_H-gene pattern in the TAK, TOU and EZZ DNAs was made using μ- and δ-specific probes (Fig. 2) plus ε- and α-specific probes (Fig. 3), and the latter two gave further evidence for gene deletions. The probes used

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in Fig. 2 were C75pl.2 (a 1.2-kb *Eco*RI fragment of a human clone, carrying the C_μ coding regions, cloned into pACYC184[8]) and M13RP2 (a 1.5-kb *Eco*RI–*Pst*I fragment of a human clone containing part of the δ gene cloned into M13mp8). All five DNA samples analysed with the μ probe possessed 17-kb hybridizing *Bam*HI fragments (Fig. 2*a*) but in addition TAK14 and TAK3 showed a hybridizing band of 8 kb. The occurrence of this latter band in TAK3 (who is thought to be homozygous for the $\mathrm{C_H}$ locus[2]) is surprising; the most obvious conclusion must be that a duplication of μ has occurred in TAK DNA. No such putative duplication has occurred in the homozygous individuals EZZ or TOU, where we find only the 17-kb *Bam*HI restriction fragment (Fig. 2*a*, lane 5). A completely normal pattern of hybridization between TAK and TOU DNAs was found when we used the δ probe (Fig. 2*b*), as each of these DNAs display a 11-kb hybridizing *Bam*HI fragment found in 9 out of 10 control DNAs analysed (latter data not shown).

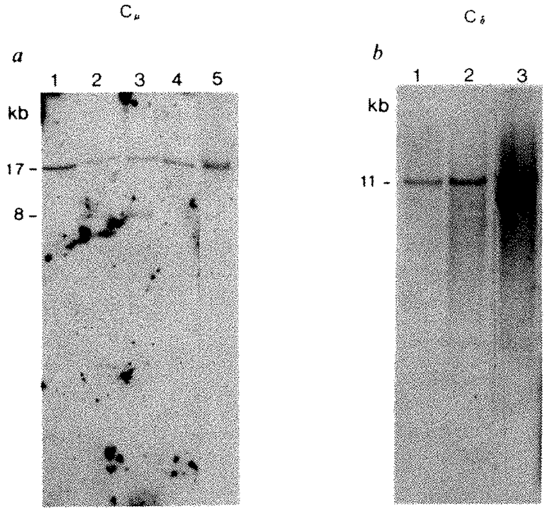

Fig. 2 Southern hybridization of Cμ and Cδ probes to genomic DNA from various sources. The DNA digests were: *a*, probe: μ; slot 1, LAT; slot 2, TAK14; slot 3, TAK3; slot 4, placenta; slot 5, EZZ. *b*, Probe: δ; slot 1, TAK3; slot 2, TAK14; slot 3, TOU. **Methods:** Genomic DNA was digested to completion with *Bam*HI, fractionated and blotted from 0.8% agarose gels onto cellulose nitrate and hybridized (as in Fig. 1) to nick-translated C75pl.2 (*a*) and M13RP2 (*b*) probes. Phage λ DNA cut with *Hin*dIII was used as molecular weight markers.

The ε and α genes in the various human DNAs were analysed using as hybridization probe for ε, the ε1.2BP25 clone (a 2.1-kb *Bam*HI–*Pst*I fragment, derived from the clone ε1.2, containing the entire human ε coding region[9]) and as an α probe the clone α2XP8 (a 600-base *Xho*I–*Pst*I fragment of an α-gene cloned in M13mp8[10]). The restriction pattern most frequently seen for ε-gene hybridization in human DNA (Fig. 3) is the presence of three *Bam*HI fragments (2.7, 6 and 9 kb)[9,11,12]. This pattern is exemplified in Fig. 3*a*, lane 3, by the DNA sample LAT. A frequently observed polymorphism is the presence of a fourth, 7.5-kb band[9], which is present in DNA sample BOU (Fig. 3*a*, lane 5). It has recently been demonstrated that the 2.7-kb fragment carries the active ε gene[9,11,12] whereas the 6- and 9-kb fragments represent pseudogenes called $\psi\varepsilon 1$ and $\psi\varepsilon 2$, respectively[10,11]. When we examined the patterns of hybridization of the ε probe to both TAK and TOU DNAs we again found evidence for gene deletion (Fig. 3*a*, lanes 1, 4). Both TAK3 and TOU DNAs show the presence of both the active ε gene and $\psi\varepsilon 2$ but no $\psi\varepsilon 1$. This observation is consistent with the deletion of the latter from these genomes because this gene is present in other DNAs (see, for example, LAT and BOU, lanes 3 and 5) including TAK14 who is heterozygous for the abnormality (Fig. 3*a*, lane 2).

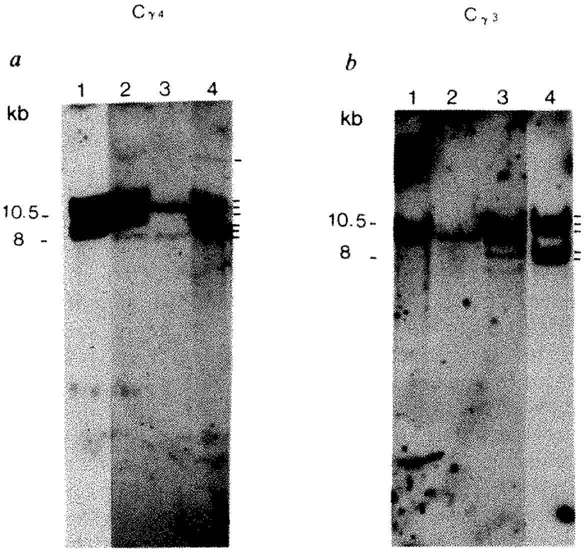

Fig. 1 Southern hybridization of Cγ3 and Cγ4 probes to DNAs from individuals showing serum immunoglobulin abnormalities. *a*, Probe: γ4; slot 1, LAT; slot 2, TAK14; slot 3, TAK3; slot 4, BOU. *b*, Probe: γ3; slot 1, TOU; slot 2, TAK3; slot 3, TAK14; slot 4, placental DNA.
Methods: DNA was digested to completion with *Bam*HI, the digests were fractionated on 0.8% agarose gel, and the DNA was transferred to cellulose nitrate filters[14]. LAT and BOU individuals were described previously[15]. The hybridization was carried out for 2 days at 65 °C using 6 × SSC, 0.1% SDS, 0.2% Ficoll 400, 0.2% bovine serum albumin (BSA), 0.2% polyvinylpyrrolidone (PVP) and 50 μg ml^{-1} sonicated, denatured, salmon sperm DNA[16,17], followed by washes in 1 × SSC, 0.1% SDS at 65 °C before autoradiography at −70 °C with prefogged X-ray film[18]. Probes were nick-translated to a specific activity of $\sim 10^8$ c.p.m. per μg. Size estimations (shown on the left of each panel) were made using λ cut with *Hin*dIII and the lines on the right of each panel signify location of restriction fragments discussed in the text.

The α-gene hybridization pattern observed using the α2XP8 clone with TAK and TOU is shown in Fig. 3*b*. In this experiment we used a sample designated TAK92 (a non-blood relative of TAK3) as the control DNA: hybridization of the α probe with TAK92 (Fig. 3*b*, lane 3) shows two hybridization components (2 and 1.2 kb) in common with European DNAs tested simultaneously (latter data not shown). Partial nucleotide sequencing data[10] indicate that the 2- and 1.2-kb fragments contain $\alpha 2$ and $\alpha 1$ coding sequences, respectively. The α-gene hybridization to DNA from TOU and TAK3 (Fig. 3*b*, lanes 1, 2) showed, in both cases, that the smaller of the hybridization components was absent, consistent with the presence of the $\alpha 2$ gene and deletion of the $\alpha 1$ gene from the genome of TAK3 and TOU individuals.

The Southern hybridization results presented here show that Tunisian individuals who exhibit simultaneous absence of IgG1, IgG2, IgG4 and IgA1 subclasses carry deletions for three γ genes (presumably $\gamma 1$, $\gamma 2$ and $\gamma 4$), a pseudo-ε gene ($\psi\varepsilon 1$) and an α gene presumed to be $\alpha 1$. These gene deletions account for the lack of specific immunoglobulin expression in these individuals. The precise location of the gene deletion in these people need not necessarily be identical but it does seem likely that switch or S-sequences are the key to the deletion event. The S-sequences are groups of short tandemly repeated units which are probably involved in the mechanism of the class switch and have been implicated in the formation of a mouse myeloma deletion mutant[13]. A likely hypothesis to explain the deletions described here is that illegitimate recombination has occurred within the germ line between non-adjacent S-sequences with resulting deletion of the intervening $\mathrm{C_H}$ genes. Presumably, such deletions occasionally occur in all human populations but would not normally be detected because they do not seem to be harmful (the person designated TAK3, for instance, is a healthy 75-yr-old woman). However, in populations where frequent consanguinity occurs the probability of

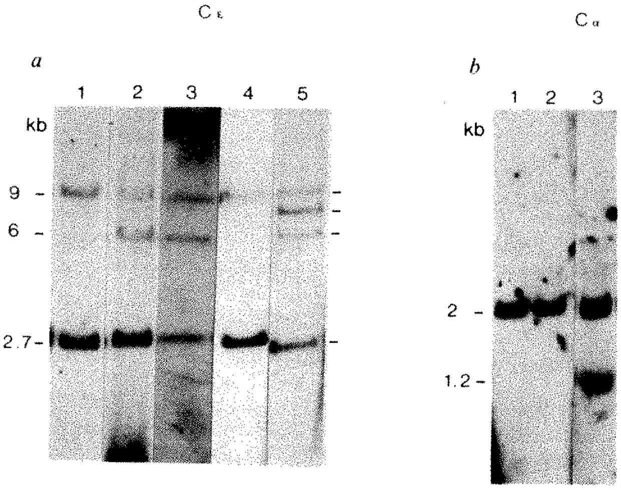

Fig. 3 Southern blot hybridization of Cε and Cα probes to genomic DNA digested with *Bam*HI (*a*) or *Pst*I (*b*). The DNA digests were: *a*, Probe: ε; slot 1, TAK3; slot 2, TAK14; slot 3, LAT; slot 4, TOU; slot 5, BOU. *b* Probe: α; slot 1, TOU; slot 2, TAK3; slot 3, TAK92.
Methods: Complete digests were separated on 0.8% agarose (*a*) or 1.4% agarose (*b*), blotted and hybridized (as in Fig. 1) with Cε probe (*a*) or Cα probe (*b*). The size of fragments (given on the left of each panel) were estimated relative to λ phage DNA cut with *Hin*dIII. The lines on the right of *a* mark the positions of fragments discussed in the text.

homozygosity.increases and such individuals are readily detected during random immunoglobulin haplotype screening.

Finally, the consistent deletion pattern of C_H genes in TAK, TOU and EZZ allows a link between the absence of C_H genes and the overall ordering of the human C_H genes described in the accompanying paper[10]. Two groups of cosmid clones have been described which seem to encompass $\gamma 3$-$\gamma 1$-$\psi\varepsilon 1$-$\alpha 1$ (region A) and $\gamma 2$-$\gamma 4$-ε-$\alpha 2$ (region B)[10]. As it seems that the deletions described here include $\gamma 1$, $\gamma 2$, $\gamma 4$, $\psi\varepsilon 1$ and $\alpha 1$ genes, the most probable order for the groups of cosmid clones is 5′ region A–region B 3′ as the deletions presumably start downstream of $\gamma 3$ (region A) and end upstream of the active ε gene (region B). We are now using gene cloning studies to clarify these issues as well as the nature and localization of the points of deletion within the C_H genes.

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Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change

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Several different transforming genes have been observed in the DNA of a variety of tumours and tumour cell lines of human and rodent origin by the ability of these genes to induce morphological transformation in NIH 3T3 cells[1–5]. The transforming gene found in a human bladder carcinoma cell line, T24, is H-*ras*-1, the human homologue of the Harvey sarcoma virus oncogene (v-H-*ras*)[6–9]. In the present study we have compared the H-*ras*-1 genes cloned from T24 and normal human DNA. The H-*ras*-1 gene cloned from T24 DNA induces transformation in NIH 3T3 cells, while the same gene cloned from normal cellular DNA does not. The functionally significant difference between the transforming and normal genes appears to be a single base mutation, which produces an amino acid change in the sequence of the proteins that the genes encode.

We had previously isolated transforming sequences from NIH 3T3 cells that had been transformed with DNA from T24 bladder carcinoma cells[10]. These sequences had undergone some rearrangement during gene transfer into NIH 3T3 cells. As a result of passage in NIH 3T3 cells, the transforming gene might have undergone less readily detectable secondary changes altering its biological activity. Therefore, in order to properly compare the transforming sequences of T24 to their normal counterparts, we directly cloned the H-*ras*-1 sequences from λ libraries constructed from T24 DNA and human placental DNA. From the T24 library we obtained seven independent isolates, all of which contained a 6.2-kilobase (kb) *Bam*HI H-*ras*-1 fragment with an identical restriction endonuclease pattern. From the placental library, we obtained clones of two types: in three (P3 type), H-*ras*-1 sequences were carried on a 6.7-kb *Bam*HI fragment: in the four others (P1 type), H-*ras*-1 sequences were carried on a 8.3-kb *Bam*HI fragment (see Fig. 1). Differences in the size of the *Bam*HI fragments were expected due to restriction endonuclease fragment length polymorphism about this gene in the human population[10].

We tested all of our isolates for the ability to induce foci of morphologically transformed NIH 3T3 cells on DNA mediated gene transfer. DNA from all T24 isolates of H-*ras*-1 efficiently transformed NIH 3T3 cells, while DNA from all placental isolates failed to induce foci. This difference must be the result of differences in DNA sequence. To facilitate our search for these differences, we tested the transforming activity of chimaeric genes. The restriction endonuclease *Xba*I cleaves the *Bam*HI fragment containing the H-*ras*-1 gene into one small and one large fragment (see Fig. 1). The large and small fragments were purified from each of the three cloned genes (types T24, P1 and P3), mixed and ligated in various combinations and finally cleaved with *Bam*HI. All (and only) molecules containing the small T24 *Bam*HI/*Xba*I fragment were capable of inducing transformed foci in NIH 3T3 cells (see Table 1). To further limit the area of our search we used restriction endonuclease *Mst*II, which cleaves the small *Bam*HI/*Xba*I fragment once. The 1.7-kb *Bam*HI/*Mst*II fragment and the 0.24-kb *Mst*II/*Xba*I fragment from the T24 or P3 H-*ras*-1 genes, and the large *Xba*I/*Bam*HI fragment from the P3 gene were purified, mixed and ligated in various combinations, and then cleaved with *Bam*HI. All molecules containing the 0.24-kb *Mst*II/*Xba*I fragment from T24 induced transformed foci in

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NIH 3T3 (see Table 1). The sequence changes in this region are therefore sufficient to confer the transforming capacity on the P3 H-*ras*-1 gene. A few foci are seen in one other combination of fragments (see Table 1) but we hesitate to ascribe significance to this.

We sequenced the 245-bp *Mst* ≤ I*Xba*I fragment in its entirety from P1, P3 and T24 H-*ras*-1 genes. The sequences were identical at all but two nucleotide positions (see Fig. 1). These lie within the first coding exon of the H-*ras*-1 gene as determined by a comparison of genomic sequences to those of an H-*ras*-1 cDNA clone. (O.F. *et al.*, manuscript in preparation).

We found that P1 and P3 H-*ras*-1 genes encoded glycine (GGC) at amino acid 12, while the T24 gene encodes valine (GTC) at that position, the result of a single G→T nucleotide change. We also noted that while all three genes encode histidine at amino acid 27, the codon usage differs (see Fig. 1). Because this latter change does not alter the protein sequence we believe it is not functionally significant (see below).

The above data suggest that an amino acid substitution in the T24 H-*ras*-1 gene product generates a protein with greatly enhanced transforming activity. To test this prediction, we have introduced the T24 and placental H-*ras*-1 genes into NIH 3T3 cells using the pSV2gpt vector of Mulligan and Berg[11]. In this way, we obtained 10 xgprt$^+$ lines containing the T24 H-*ras*-1 gene and 16 lines containing the P3 H-*ras*-1 gene, as determined by Southern filter blot hybridization (see Fig. 2). All ten of the lines containing the T24 H-*ras*-1 gene appeared morphologically transformed, while only two of the lines containing the P3 H-*ras*-1 gene had any of the features we associate with morphological transformation. To measure more precisely the degree of growth transformation we examined the ability of several lines to grow in agar suspension (see Fig. 2). To correlate degree of transformation with gene expression, we measured H-*ras*-1 RNA by filter hybridization and H-*ras*-1 protein by immunoprecipitation of ^{35}S-methionine labelled cell lysates (see Fig. 2). The human H-*ras*-1 gene encodes a 21,000-MW protein (p21) which cross-reacts with antibody to the Harvey v-*ras* p21 (given by M. Furth and E. Scolnick).

Table 1 Focus induction with chimaeric H-*ras*-1 genes

(5')*Bam*HI *Xba*I	*Xba*I *Bam*HI(3')		Foci per 0.1 µg*
T24	T24		358
T24	P1		142
T24	P3		80
P1	T24		0
P1	P1		0
P3	T24		0
P3	P3		0
(5')*Bam*HI/ *Mst*II†	*Mst*II/*Xba*I†	*Xba*I/ *Bam*HI(3')†	Foci per 0.02 µg‡
T24	T24	P3	88
T24	P3	P3	2
P3	T24	P3	163
P3	P3	P3	0

The T24, P1 and P3 *Bam*HI fragment containing the H-*ras*-1 gene was cleaved with *Xba*I and the respective 5' (*Bam*HI/*Xba*I) and 3' (*Xba*I/*Bam*HI) halves of the genes (see Fig. 1) purified from agarose gels as previously described[25]. The various 5' and 3' halves were mixed in equimolar ratios in the combinations indicated above, ligated with T4 DNA ligase at a DNA concentration of 100 µg ml^{-1}, and then digested with *Bam*HI. The amount of material in the correct molecular form was then estimated by ethidium bromide staining after electrophoresis in agarose gels.

* Approximately 0.1 µg of the chimaeric genes was mixed with 90 µg of NIH 3T3 carrier DNA and added to 3 plates of NIH 3T3 as a calcium phosphate precipitate as previously described[4]. Morphologically altered foci of NIH 3T3 cells were scored after 14 days.

† The 1.7-kb *Bam*HI/*Mst*II and the 0.24-kb *Mst*II/*Xba*I fragments were purified from the T24 and P3 *Bam*HI fragments (see Fig. 1), mixed in equimolar ratio with the 4.8-kb P3 *Xba*I/*Bam*HI fragment, ligated with T4 DNA ligase at a DNA concentration of 100 µg ml^{-1} and then cleaved with *Bam*HI. The amount of material in the correct molecular form was then estimated by ethidium bromide staining after gel electrophoresis.

‡ Approximately 20 ng of the chimaeric genes were mixed with 180 µg of NIH 3T3 carrier DNA and added to six plates of NIH 3T3. Foci were scored after 14 days.

In these studies, the only difference we can discern between the expression of the P3 and T24 H-*ras*-1 genes is the potency of their respective protein products. Highly transformed cell lines containing the T24 H-*ras*-1 gene (XT1 and XT3) contain far less *ras* p21 than do minimally transformed lines expressing the normal *ras* gene (XP1, XP3). We estimate from this that the T24-encoded *ras* p21 is at least 100 times more potent at inducing growth transformation than the P3-encoded *ras* p21.

We note that one line (XP2) containing the P3 H-*ras*-1 gene and expressing the highest levels of *ras* p21 does grow in agar to a significant extent. Thus it appears that normal H-*ras*-1 can mediate transformation if the gene product is overproduced. Other workers have reported that a normal allele of H-*ras*-1 acquires transforming activity when physically linked to a high efficiency retroviral promoter[12]. However, very high levels of the normal gene product are required to achieve the degree of

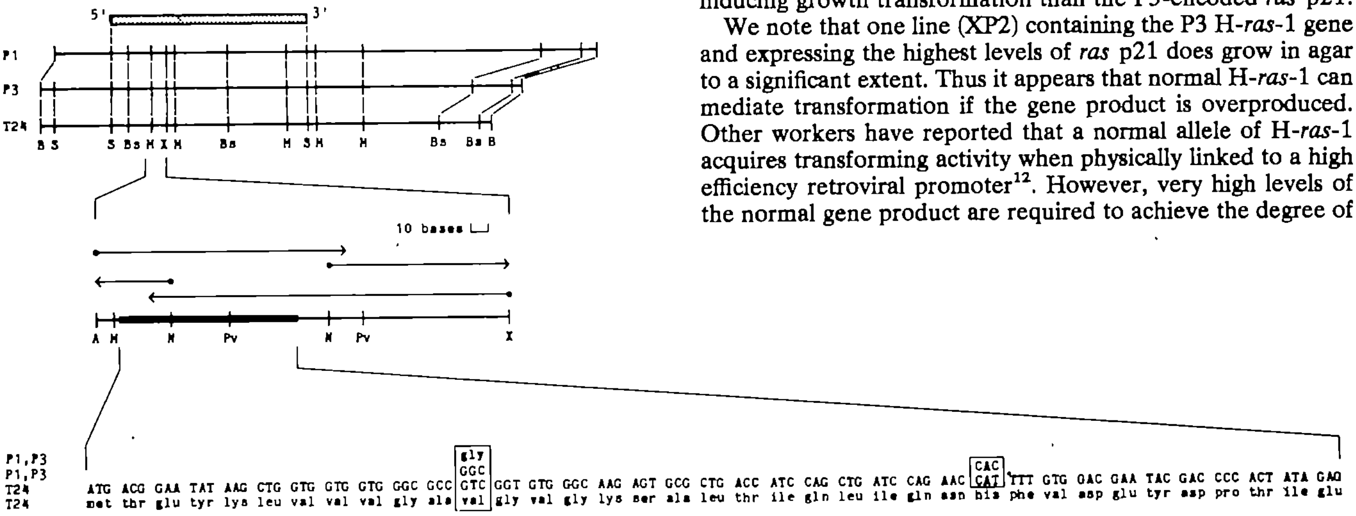
5'
3'
P1
P3
T24
B S S Bs M X M Bs M S M M Bs Bs B
10 bases
A M N Pv N Pv X
P1,P3
P1,P3
T24
T24
gly
GGC
CAC
ATG ACG GAA TAT AAG CTG GTG GTG GTG GGC GCC GTC GGT GTG GGC AAG AGT GCG CTG ACC ATC CAG CTG ATC CAG AAC CAT TTT GTG GAC GAA TAC GAC CCC ACT ATA GAG
met thr glu tyr lys leu val val val gly ala val gly val gly lys ser ala leu thr ile gln leu ile gln asn his phe val asp glu tyr asp pro thr ile glu

Fig. 1 Structural differences between human H-*ras*-1 genes. The *Bam*HI fragment containing the H-*ras*-1 gene was cloned from placental DNA and T24 DNA libraries constructed in the L47.1 cloning vector[13]. Phage libraries were constructed by the method of Hohn and Murray[14] and screened without prior amplification by the method of Benton and Davis[15]. Restriction maps of the three gene types (P1 and P3 from placenta, and T24 from T24 cells) are shown above. In the region of the 2.9-kb *Sst*I fragment (shaded area) which contains a complete H-*ras*-1 gene (unpublished data) the restriction maps are similar. The 245-bp *Mst*II/*Xba*I fragment, which contains the functionally significant structural differences, was sequenced by the method of Maxam and Gilbert[16] in entirety for all three genes. The sequencing strategy is shown in the middle panel. Restriction fragments were labelled at their 3' ends with the large fragment of DNA polymerase I[17]. All regions were sequenced at least once on both strands. The bold line indicates the first coding exon of 37 amino acids, determined by sequencing a T24 H-*ras*-1 cDNA clone (O.F. *et al.*, manuscript in preparation). In the bottom panel is shown the DNA sequence of T24 in this coding region and the predicted amino acid sequence. Where P1 and P3 differed from T24 are enclosed in boxes. Restriction endonucleases used were *Ava*I (A), *Bam*HI (B), *Bst*EII (Bs), *Mst*II (M), *Nar*I (N), *Pvu*II (Pv), *Sst*I (S) and *Xba*I (X).

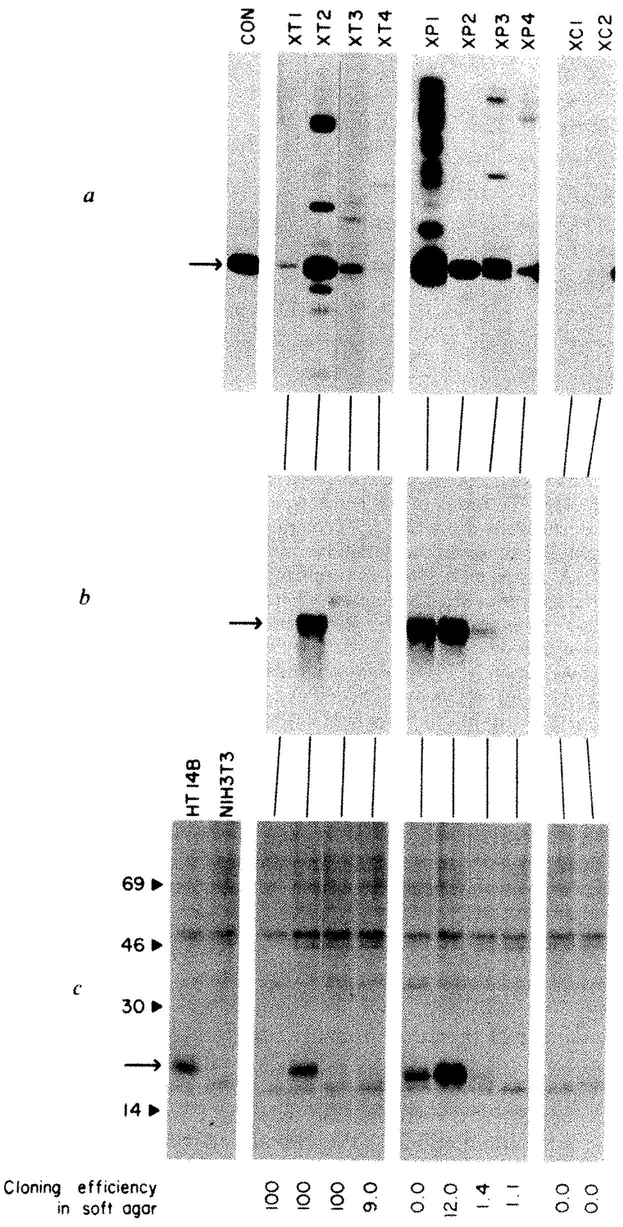

Fig. 2 Expression of normal and transforming H-*ras*-1 genes after co-transfer into NIH 3T3 cells. NIH 3T3 cells containing the human H-*ras*-1 gene cloned from T24 (XT1 . . . XT4) or from placental DNA (XP1 . . . XP4) were obtained by co-transfer[18] after selection for expression of the pSV2gpt vector[11] added in the presence of an excess of the purified *Bam*HI fragments containing the H-*ras*-1 gene. Cells were analysed for H-*ras*-1 gene sequences (*a*), expression of H-*ras*-1 transcripts (*b*), expression of the p21 *ras* gene product (*c*), and cloning efficiency in soft agar (bottom). XC1 and XC2 are NIH 3T3 cells containing the pSV2gpt vector but no human H-*ras*-1 gene. HT14B are NIH 3T3 cells transformed with unintegrated Harvey sarcoma viral DNA. *a*, 6 μg DNA from the indicated cell lines were cut with *Sst*I, electrophoresed in 1.0% agarose gels, filter•blotted according to Southern[19] and filters hybridized with 2.9-kb *Sst*I fragment purified from the human H-*ras*-1 gene (see Fig. 1) and nick translated with ^{32}P (ref. 20) to a specific activity of 3×10^8 cpm μg^{-1}. Filter hybridization and washing conditions were at 72 °C under stringent conditions as previously described[4]. Filters were autoradiographed at −70 °C for 15 h with XR5 film and intensifying screens. Lane labelled CON represents 100 ng of purified 2.9-kb *Sst*I fragment. The presence of the 2.9-kb *Sst*I fragment (arrow) indicates the presence of at least one functional copy of the H-*ras*-1 gene. Other hybridizing fragments represent integration events in which one or both of the *Sst*I sites flanking the H-*ras*-1 gene were destroyed. *b*, Cytoplasmic RNA from the indicated cell line was prepared[21] and 20 μg were electrophoresed in 1.0% agarose formaldehyde gels as previously described[10], filter blotted and hybridized according to Thomas[22] except that dextran sulphate was omitted. The hybridization probe was as described above. The arrow indicates the position of the 1.1–1.2-kb H-*ras* mRNA. *c*, Indicated cells were labelled for 6 h in methionine-free Dulbecco's medium containing 5% dialysed serum and 100 μCi ml^{-1}, 80 Ci mmol^{-1} ^{35}S-methionine. Washed cells were lysed in 1% Triton, 0.5% deoxycholate in phosphate-buffered saline containing 2 μg aprotinin (Sigma) and 1 mM phenylmethylsulphonylfluoride, and centrifuged at 100,000*g* for 12 h. Supernatants were preabsorbed with goat anti-rat IgG and *Staphylococcus aureus* protein A. Immunoprecipitation was performed for 5 h at 4 °C with anti-v-H-*ras* p21 monoclonal antibody, the generous gift of E. Scolnick and M. Furth. After addition of goat anti-rat IgG, immune complexes were absorbed to protein A. Pellets were boiled in SDS sample buffer[23] and analysed by SDS-polyacrylamide gel electrophoresis by the method of Blattler *et al.*[24]. Proteins were visualized after fluorography by exposure for 30 h to Kodak XR5 film. The arrow marks the position of migration of v-H-*ras* p21. Arrowheads are size markers ($\text{MW}\times 10^{-3}$) from ^{14}C-labelled standards (bovine serum albumin, ovalbumin, carbonic anhydrase and lysozyme; Amersham). Cloning efficiency in soft agar was determined by plating 10^5 cells in 5-cm dishes containing 0.35% Difco agar and Dulbecco's medium with 10% calf serum. 14 days later, numbers of colonies with greater than 10 cells were tabulated for each cell line by examination of six 2-mm diameter microscopic fields.

growth transformation mediated by low levels of an altered *ras* gene product.

We and others have shown transforming *ras* genes are present in many different human tumour cell lines[6-9]. We speculate that the activation of *ras* genes in these other cell lines is the consequence of somatic mutations in coding regions. If such mutations do occur in human tumour cells, the altered protein products might be immunogenic in their human hosts. The presence, therefore, of antibodies against *ras* proteins in humans may be diagnostic for certain forms of cancer.

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Note added in proof: Substantially similar findings were recently reported for the transforming genes of T24 (ref. 26) and EJ (ref. 27), another bladder carcinoma cell line which we believe has arisen from T24 (M.W., M.G., J. Fogh, N. Dracopoli and M. Pollack, unpublished data). Most importantly, the predicted amino acid sequences are all in agreement. We note the following differences in the critical 245-bp *Mst*II/*Xba*I fragment: (1) a *Mst*II cleavage site 9 bases 5′ to the ATG start codon is predicted and present in the genes we have sequenced, but not predicted by the published sequences of the other groups. (2) A *Nar*I cleavage site 104 bases 3′ to the ATG start codon is predicted and present in the genes we have sequenced, but not found in the sequence published by Tabin *et al.*[27]. (3) An altered histidine codon is not observed for the normal gene sequenced by the other groups.

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A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia

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The transforming genes of oncogenic retroviruses are homologous to a group of evolutionary conserved cellular *onc* genes[1]. The human cellular homologue (c-*abl*) of the transforming sequence of Abelson murine leukaemia virus (A-MuLV) was recently shown[2] to be located on chromosome 9. The long arm of this chromosome is involved in a specific translocation with chromosome 22, the Philadelphia translocation (Ph^1), t(9; 22) (q34, q11), which occurs in patients with chronic myelocytic leukaemia (CML)[3–5]. Here we investigate whether the c-*abl* gene is included in this translocation. Using c-*abl* and v-*abl* hybridization probes on blots of somatic cell hybrids, positive hybridization is found when the $22q^-$ (the Philadelphia chromosome), and not the $9q^+$ derivative of the translocation, is present in the cell hybrids. From this we conclude that in CML, c-*abl* sequences are translocated from chromosome 9 to chromosome $22q^-$. This finding is a direct demonstration of a reciprocal exchange between the two chromosomes[6] and suggests a role for the c-*abl* gene in the generation of CML.

The human c-*abl* sequences represent a cellular homologue of the transforming component of A-MuLV. This retrovirus is a recombinant between Moloney MuLV and mouse cellular c-*abl* sequences[7] and induces lymphoid tumours on *in vivo* inoculation of the mouse[8,9]. The major A-MuLV translational product has been identified as a poly-protein, $\mathrm{P120}^{gag\text{-}abl}$, consisting of amino-terminal structural proteins encoded by the M-MuLV *gag* gene, linked to an acquired cellular sequence encoded carboxy-terminal component[10,11]. This protein is one of several virus-encoded transforming proteins with tyrosine-specific protein kinase activity[12–15]. Similar oncogenic sequences of Harvey and Kirsten sarcoma virus are homologous to transforming sequences (c-Ha-*ras*, c-Ka-*ras*) isolated from human bladder and lung carcinoma cell lines[16–18]. Both these sequences induce transformation of mouse NIH 3T3 cells after transfection, establishing that the human genes have potential transforming activity. Recently, the human c-*abl* gene has been cloned in cosmids[19]. Using v-*abl* DNA as a probe, several clones containing overlapping sequences representing the entire c-*abl* gene were isolated from a human lung carcinoma cosmid library. The restriction enzyme map of the human v-*abl* cellular homologue, presented in Fig. 1, identifies areas of the gene which hybridize to v-*abl* sequences. The gene is distributed over a region of 40 kilobases (kb) of human DNA and contains multiple intervening sequences. On transfection of Rat-2 cells with the c-*abl* cosmids, no transforming activity was detected, not unexpectedly, as none of the cosmid clones tested contained the entire c-*abl* gene[19].

By Southern blot analysis of a series of somatic cell hybrids, the human c-*abl* gene has been localized on chromosome 9[2]. This finding is of interest because of the involvement of the long arm of chromosome 22 (band 22q11) in a specific translocation with the long arm of chromosome 9 (band 9q34), the Philadelphia translocation (Ph^1), occurring in human CML[3,4]. The abnormal chromosomes are designated $9q^+$ and $22q^-$; of these, the $22q^-$ chromosome is observed in 92% of CML cases[5]. We investigated the chromosomal location of the human c-*abl*

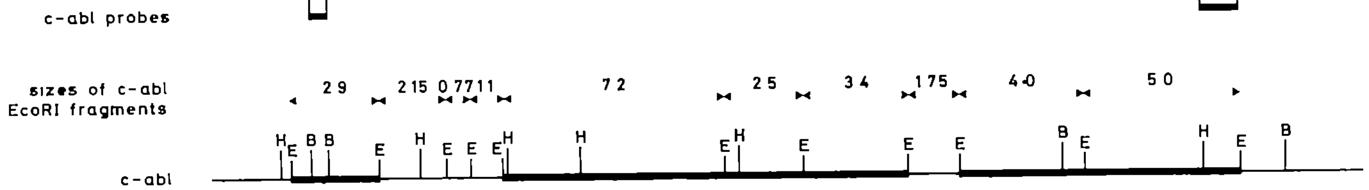

Fig. 1 Restriction enzyme map of the human c-*abl* region[19]. The upper line of the figure shows the *Bam*HI subclone of A-MuLV; the hatched box presents the long terminal repeat, the solid bar the acquired cellular sequences. Directly beneath the A-MuLV genome, a subgenomic *Pst* 1.7-kb fragment, used as a probe in this study, is shown. Human c-*abl* DNA restriction fragments homologous to v-*abl* sequences are indicated as black boxes and those that show homology to the 1.7-kb *Pst* v-*abl* fragments are designated by P 1.7. The third line shows the human c-*abl* 0.6-kb *Bam*HI and 2.2-kb *Hin*dIII-*Eco*RI probes, which hybridize to 5′ and 3′ c-*abl Eco*RI fragments, respectively. The sizes of all *Eco*RI c-*abl* fragments are indicated on the fourth line. The bottom line represents the restriction enzyme map of the human c-*abl* gene. Restriction enzymes include *Bam*HI (B), *Hin*dIII (H) and *Eco*RI (E). A more detailed characterization of the human c-*abl* locus will be published elsewhere[19].

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Table 1 Human chromosome content of human–mouse and human–Chinese hamster somatic cell hybrids

Hybrid	Human chromosomes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	9q⁺	22q⁻	Human isoenzyme AKl	Ref.
PgMe-25NU	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	26
10CB-23B	–	–	–	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	6
14CB-21A	–	–	–	+	–	–	+	+	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	+	–	+	20
1CB-17a NU	–	–	–	–	–	–	–	+	–	–	–	+	–	+	+	–	–	–	+	–	+	–	–	–	–	+	–	6
WESP-2A	–	–	–	–	–	–	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	+	–	×

The origin and details of the initial characterization of the somatic cell hybrids are described in the references listed in the last column. PgMe-25NU and WESP-2A are hybrids obtained from fusions with mouse Pg19 and WEHI-3B cells, respectively. Chinese hamster cell line E36 was used to produce hybrid clones 10CB-23B and 14CB-21A, while Chinese hamster cell line a3 was used to obtain 1CB-17aNU. Chromosome analysis was done using reverse (R) banding with acridine orange, after heat denaturation. At least 16 metaphases were analysed per cell line. The presence of human AKl activity was assayed by cellulose acetate (Cellogel) electrophoresis[27]. This test is inconclusive for the WESP-2A cell line (×), because the expression of AKl was found to be repressed in hybrids derived from fusion with WEHI-3B cells (A.H.M., G.v.K., unpublished results). Chromosome and isoenzyme analyses[22] were performed on the same batches of hybrid cells that were used for the isolation of DNA.

gene in cases of CML, where the Philadelphia translocation is present. Southern blot analyses with c-*abl* and v-*abl* probes were performed on *Eco*RI-digested DNAs from somatic cell hybrids segregating the 9q⁺ and 22q⁻ chromosomes.

The cell hybrids used here contain a full complement of mouse or Chinese hamster chromosomes and a limited number of human chromosomes. The hybrid cell lines have been obtained by fusion of cells from mouse (Pg 19 and WEHI-3B) or Chinese hamster (E36 and a3) origin with leukocytes from different CML patients and from a normal donor[6,20]. The human chromosome content of these cells is summarized in Table 1 and is based on chromosome analysis. In addition, the hybrid cells were tested for the expression of human adenylate kinase-1 (AK1) enzyme activity, a marker localized proximal in band 9q34 (ref. 21). This latter test was necessary to exclude the possibility of hidden (broken or rearranged) chromosome 9 fragments in the 22 and 22q⁻ cell lines.

Detection of the human c-*abl* restriction fragments in hybrid cell DNAs is often inconclusive using v-*abl* probes, because the human sequences are present in submolar amounts (20–50%) and also because many of the human c-*abl* restriction fragments electrophorese in close proximity with strongly hybridizing mouse or Chinese hamster fragments. To obtain molecular probes with specificity for human c-*abl* sequences, two restriction fragments were isolated from subclones of c-*abl*-containing cosmids, with homology to the presumptive 5′ and 3′ proximal *Eco*RI fragments of c-*abl*. These are 2.9 and 5.0 kb, respectively, in size (Fig. 1). After hybridization and washing to high stringency (0.1 × SSC)[12], the 5′-terminal 0.6-kb *Bam*HI probe and the 3′-terminal 1.1-kb *Hin*dIII–*Eco*RI probe cross-

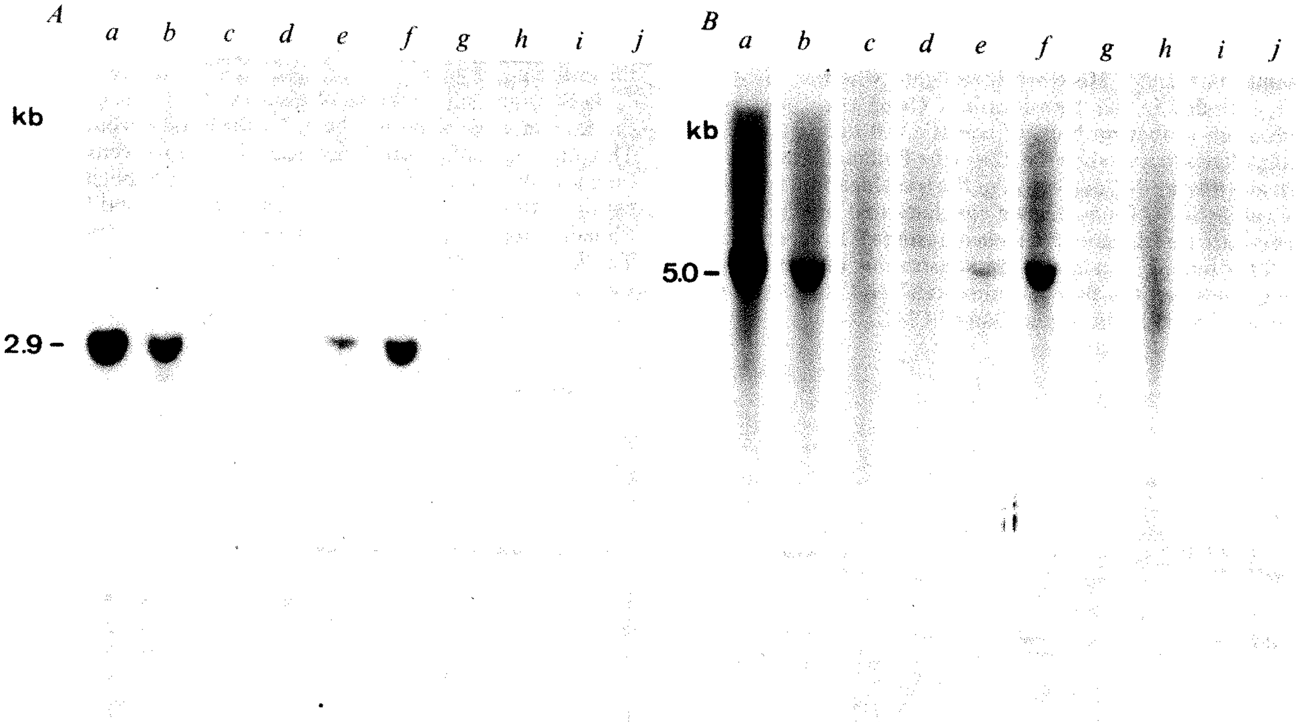

Fig. 2 Localization of human c-*abl* sequences on the Philadelphia chromosome, using hybrid cell lines and human c-*abl* probes. *A*, detection of the human 5′ end 2.9-kb *Eco*RI c-*abl* fragment in DNA from *a*, human placenta; *b*, 10CB-23B (chromosome 9); *c*, PgMe-25NU (chromosome 22q); *d*, 14CB-21A (chromosome 9q⁺); *e*, 1CN-17aNU (chromosome 22q⁻); *f*, WESP-2A (chromosome 22q⁻); *g*, mouse Pg19; *h*, Chinese hamster E36; *i*, mouse WEHI-3B; *j*, Chinese hamster a3. *B*, detection of the 3′-end 5.0-kb *Eco*RI c-*abl* fragment in DNAs as indicated in *A*(*a*–*j*). The derivations of all these cell lines and their complements of human chromosomes are summarized in Table 1.

Methods: All cell lines used in this experiment were grown in large batches (10^7–10^8 cells) and DNA was prepared as described by Jeffreys and Flavell[28]. *Eco*RI-restricted DNAs (10 μg per lane) from human placenta, hybrid cell lines, mouse and Chinese hamster fusion partners were electrophoresed on 0.7% agarose gels. *Hin*dIII and *Hin*dIII–*Eco*RI-digested λ DNAs were included as molecular weight markers (not shown). After blotting to nitrocellulose, the filters were hybridized to the 0.6-kb *Bam*HI c-*abl* (*A*) or 1.1-kb *Hin*dIII–*Eco*RI c-*abl* (*B*) restriction fragments described in Fig. 1. Hybridization and washing procedures (to 0.1 × SSC at 65 °C) were carried out according to the method of Bernards and Flavell[23].

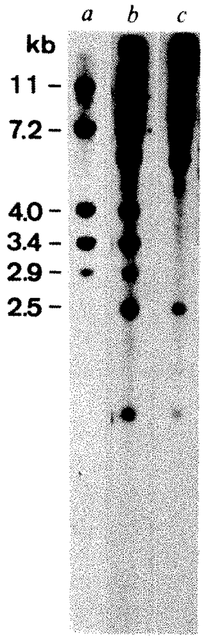

Fig. 3 Localization of human c-*abl* sequences on the Philadelphia chromosome, using a $22q^-$ somatic cell hybrid and a v-*abl* probe. *Eco*RI-digested DNAs (10 μg) from human placenta (*a*), hybrid WESP-2A (*b*) and mouse WEHI-3B cells (*c*) were hybridized with the 1.7-kb *Pst* v-*abl* fragment (Fig. 1), as described in Fig. 2 legend. After hybridization, the filters were washed to 1 × SSC at 65 °C. Molecular weights of human c-*abl* fragments were deduced from co-electrophoresed *Hin*dIII and *Hin*dIII–*Eco*RI-digested λ DNA markers.

hybridize to a very low extent with mouse or hamster c-*abl* sequences. Figure 2*A* shows an example of a hybridization experiment with the 0.6-kb *Bam*HI probe. This Southern blot illustrates hybridization of *Eco*RI-restricted DNAs of hybrid cell lines containing chromosomes 22, 9, $9q^+$ or $22q^-$. As controls, hybridization of the probe with human placenta DNA and DNA from the mouse and Chinese hamster fusion partners is shown. It is clear that the 2.9-kb *Eco*RI fragment, detected in human placenta DNA, is also present in the lanes containing DNA from the hybrid cell lines 10 CB-23B (chromosome 9), 1CB-17a NU and WESP-2A (both containing chromosome $22q^-$). The band is not detected in lanes containing DNA from PgMe-25Nu (chromosome 22), 14CB-21A (chromosome $9q^+$), Pg19 and WEHI-3B (mouse controls) or E36 and a3 (Chinese hamster controls). Analogous results are obtained when the same *Eco*RI-digested DNAs are hybridized to the 3′-terminal 1.1-kb *Hin*dIII–*Eco*RI probe (Fig. 2*B*). The 5.0-kb *Eco*RI fragment is detected only in DNA from human placenta and from hybrid cell lines containing chromosome $22q^-$ or 9.

The above results show that both the 5′ and 3′ ends of the c-*abl* gene are translocated to chromosome $22q^-$. Because all other c-*abl* *Eco*RI fragments, which hybridize to v-*abl* sequences, are flanked by the 2.9-kb and 5.0-kb *Eco*RI fragments, it seems highly probable that these fragments are also included in the translocation to the Philadelphia chromosome. To test this possibility directly, hybridization was performed using a 1.7-kb *Pst* v-*abl* probe (Fig. 1). Because of the problems with v-*abl* probes indicated above, only WESP-2A, the hybrid containing the most $22q^-$ sequences (50% of the molar amount), was tested. As shown in Fig. 3, the viral probe detects human *Eco*RI c-*abl* fragments of 11, 7.2, 4.0, 3.4, 2.9 and 2.5 kb (weakly). Of these fragments, the 11-kb band has been shown to map outside the main human c-*abl* locus[19] and will not be considered here. The human 2.9-, 3.4-, and 4.0-kb c-*abl* fragments are readily detected in the WESP-2A DNA. In contrast, the 7.2-kb *Eco*RI fragment can only be seen in a short exposure of this filter (not shown), due to spill-over of radiation from strongly hybridizing mouse c-*abl* fragments in this area. The 2.5-kb *Eco*RI human c-*abl* fragment co-migrates with a mouse fragment of similar size and thus cannot be identified in this analysis.

The hybrid cell lines containing the $9q^+$ and $22q^-$ chromosomes examined in the present study, were obtained from fusion experiments with CML cells from three different individuals. Therefore, we conclude that in the Philadelphia translocation a fragment of chromosome 9 is translocated to chromosome $22q^-$ and that this fragment includes the human c-*abl* sequences. This finding establishes that the translocation is reciprocal, a general assumption which is now demonstrated unequivocally. Moreover, the data map the human c-*abl* sequences distal to AK1 (not translocated to $22q^-$, 6, 20) on chromosome 9. The most interesting aspect is that it raises the possibility of involvement of the human c-*abl* gene in the generation of CML.

In principle, the chromosomal translocation associated with CML could lead to elevated levels of c-*abl* expression which, by analogy to the c-Ha-*ras* gene in bladder carcinoma, would induce malignant transformation[24]. Elevated levels of c-*abl* expression could be the result of coupling of the gene to an enhancer sequence present on chromosome 22 or, alternatively, the gene could be linked to a strong promoter of another gene. To test these possibilities, we have initiated studies to clone the c-*abl* gene from the $22q^-$ chromosome using WESP-2A DNA and a cosmid vector system. Finally, it is of interest that in some CML patients variant Ph^1 translocations are observed, in which the participation of chromosome 9 cannot be detected by classical cytogenetic analysis[3,5]. In another group of CML patients the Ph^1 translocation appears to be completely absent[25]. We are now investigating whether the c-*abl* gene is translocated to chromosome 22 in these cases also.

These studies were initiated as part of a collaborative effort with W. F. Bodmer; his helpful discussions throughout the work are greatly appreciated. We also thank F. Grosveld for important contributions to this study, R. A. Flavell for useful suggestions, Ton van Agthoven, Gail T. Blennerhassett and Pam Hansen for technical assistance and Ad Konings and Rita Boucke for help with the preparation of the manuscript. The work was supported by the Netherlands Cancer Society (Koningin Wilhelmina Fonds) and under NCI PHS contract NOI-CO-75380. C.R.B. is a recipient of a fellowship from the Deutsche Forschungsgemeinschaft.

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Abnormal RNA processing due to the exon mutation of β^E-globin gene

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As is typical of all β-thalassaemias[1], the erythroid cells of individuals with the variant haemoglobin E ($\alpha_2\beta_2{}^{26\mathrm{Glu}\rightarrow\mathrm{Lys}}$) exhibit a quantitative deficiency in their content of β-globin (in this case β^E-globin) and its messenger RNA[2,3]. To determine the molecular basis of this phenotype, we have investigated the structure and expression of cloned β^E-globin genes. We report here that the complete nucleotide sequence of a β^E-gene revealed the expected GAG → AAG change in codon 26 but no other mutations. Expression of β^E-globin genes introduced into HeLa cells revealed two abnormalities of RNA processing: slow excision of intervening sequence-1 (IVS-1) and alternative splicing into exon-1 at a cryptic donor sequence within which the codon 26 nucleotide substitution resides. These results demonstrate a disturbance in the expression of the β^E-gene attributable solely to the exon mutation—a novel mechanism for gene dysfunction.

We first examined the nucleotide sequences of β^E-globin genes to determine whether there were two mutations, one in codon 26 and another elsewhere in the gene causing a reduction of mRNA level, or only a single mutation. We studied two different cloned β^E-globin genes that were distinguishable by their intragenic DNA polymorphisms[4]. Underproduction of β^E-globin in individuals bearing either of these two gene types suggests a close relationship between the protein/mRNA deficiency and the exon mutation. The complete DNA sequence of one of the β^E-globin genes (β_1^E), determined by the method of Maxam and Gilbert[5], revealed the expected codon 26 change (GAG → AAG) and four previously identified intragenic polymorphisms[4], but no other substitutions. Partial DNA sequencing of the other β^E-gene (β_2^E) also showed the codon 26 change and a single intragenic polymorphism[4]. Unless a common extragenic mutation were associated with these apparently independently derived β^E-genes, these sequence data indicate that the codon 26 change must be directly related to the underproduction of β^E-mRNA.

The expression of the cloned β^E-globin genes linked to Simian virus 40 (SV40) sequences in a miniplasmid[6] was studied after their introduction into HeLa cells. In this transient expression system, transcripts of the normal β-globin gene are efficiently synthesized and appropriately processed[6-8]. In all experiments we compared transcripts of the two β^E-globin genes with those of a normal gene. Cells were collected either 24 or 48 h after transfection and RNA was analysed by Northern blot analysis[9] and S_1 nuclease mapping[10] using a variety of labelled DNA probes. Both β^E-globin genes directed synthesis of near normal amounts of 9S β-globin RNA (not shown). S_1 nuclease mapping of total cell, or fractionated cytoplasmic and nuclear, RNAs revealed two striking abnormalities of β^E-RNA processing. First, the steady-state level of RNA containing IVS-1 sequences was increased (Fig. 1). Second, a fraction of β^E-transcripts was alternatively spliced (Figs 1, 2); in our hybridization conditions this abnormal product represented 5–8% of the total, a value confirmed by primer extension analysis using an exon-2 fragment.

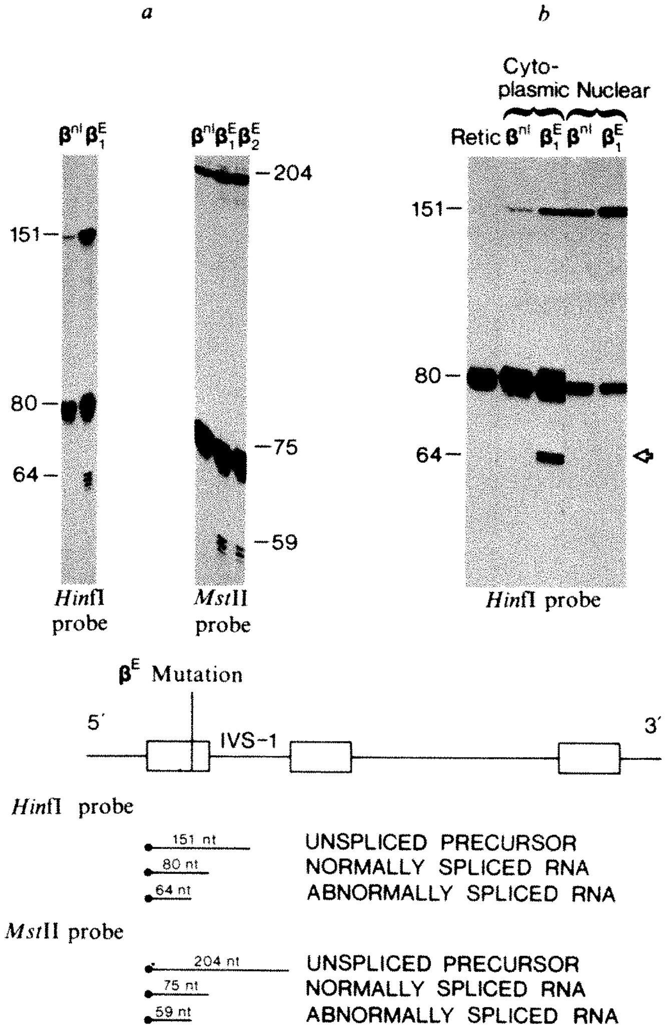

Fig. 1 Slow and alternative processing of β^E-RNA transcripts. β^E-globin genes of frameworks 1 (β_2^E) and 3 (β_1^E)[4,16] were cloned in bacteriophage Charon 28 as described previously and subcloned as a 3.7-kilobase (kb) *BglII/PstI* fragment in the miniplasmid πSV40plac[6]. These β^E-gene subclones and a normal (nl) β-globin gene oriented identically in the same expression vector, were transfected to subconfluent HeLa cells as described by Treisman *et al.*[6]. Total cellular RNAs (*a*) were isolated by repeated guanidine HCl extractions and phenol extraction at 24 h (left) and 48 h (right) after transfection. Cytoplasmic and nuclear RNA fractions (*b*) were prepared from cells 24 h after transfection as described by Favaloro *et al.*[17]. The 3′-end-labelled, single-stranded DNA probes indicated were prepared from β^E-globin gene DNA by filling in 5′-overhangs with ^{32}P-dXTPs (each at 600 Ci mmol^{-1}) using the Klenow function of *Escherichia coli* DNA polymerase I and isolation of the anti-sense strand[5]; 25 μg total or cytoplasmic and 10 μg nuclear RNAs plus DNA probe were denatured in hybridization buffer[17], incubated at 37 °C for 20 h, and then treated with 1,000 U ml^{-1} S_1 nuclease (Sigma) at 37 °C for 40 min. Reticulocyte globin mRNA (6.5 ng) was assayed in parallel as a control. Protected DNAs were electrophoresed in urea–8% acrylamide gels at 2,000 V for 2 h; the gels were then exposed to X-ray film with intensification. Hybridization of the RNA and DNA probe at 51 °C rather than 37 °C led to a marked reduction in protected fragments 59 or 64 nucleotides (nt) long due to instability of short RNA–DNA hybrids at this temperature in formamide buffer (data not shown). Identical S_1 nuclease mapping results (shown above) were obtained with probes prepared from both normal and β^E-gene DNA. Note the presence of alternatively or abnormally spliced RNA in the β^E-RNA samples and the 3–5-fold increase in unspliced material relative to normal. The 64-nucleotide DNA fragment was clearly visible in the nuclear sample on the right on the original autoradiogram, as indicated by the open arrow.

Alignment of the novel protected DNA fragment observed in these S_1 mapping experiments with sequencing tracts of the probe assigned the alternative splice site to an exon-1 donor-like[11,12] sequence, GTG↓GTAAGG, that spans the codon 26 mutation itself (Fig. 2). The normal sequence of this portion of exon-1, GTGGTGAGG, resembles donor (or 5′) splice sites[11,12]. This site is not used in splicing normal gene transcripts

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(Fig. 1), but is employed in processing RNA transcripts of three different β-thalassaemic genes having substitutions in or near the IVS-1 splice junction (R. A. Treisman *et al.*, in preparation). Activation of this cryptic donor site by the β^E mutation is selective, however, in that additional cryptic sites used in these latter cases are not used to process β^E-transcripts. The cryptic splicing site generated by the codon 26 mutation apparently has sufficiently enhanced affinity with the splicing apparatus to allow it to compete with the normal donor site and retard IVS-1 excision. Additional S_1 nuclease mapping studies (not shown) revealed use of the normal IVS-1 acceptor and IVS-2 donor and acceptor sites in processing β^E-transcripts. The alternatively spliced β^E-RNA cannot encode normal globin due to loss of a portion of the exon as well as production of a downstream in-phase terminator in the processed product. Thus, the HeLa cell experiments demonstrated that β^E-precursor RNA is processed both slowly and alternatively (Fig. 3).

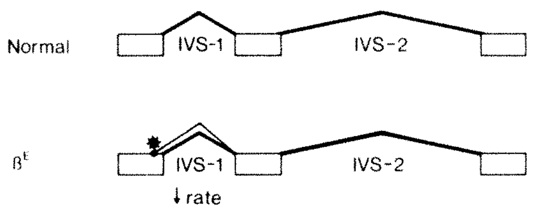

Fig. 3 Splicing patterns of normal and β^E transcripts in HeLa cells. Normal RNA is spliced exclusively from the ends of the exons as depicted. IVS-1 of β^E-RNA transcripts is removed slowly and alternative splicing into exon-1 near the β^E-mutation (asterisk) occurs. We have used the compilations of normal sequences of donor sites reported by Chambon[11] and Mount[12] to estimate the relative affinities of potential splicing sites. The relevant sequences seem to include three nucleotides preceding the invariant GT and four nucleotides thereafter. We have merely summed the number of genes reported in each compilation with a specific nucleotide at each of these positions for the sequences of the normal 5'-IVS-1 splice site, and the normal and β^E cryptic sites; using the listing of Chambon[11], the sums are 489, 448, and 468, respectively. A 'score' of 583 is the maximum possible. From the listing of Mount[12], values of 758, 577, and 622 are obtained with a maximum of 913. Overall, the codon 26 substitution makes the cryptic site of the normal more favourable as a splice site, although still not as strong as the normal site.

The *in vivo* situation in erythroid cells may be more complex than that in HeLa cells due to potential quantitative differences in mRNA metabolism. In total bone marrow RNA of one haemoglobin E homozygote, we have found little, if any, alternatively spliced steady-state β-RNA (not shown). However, in other thalassaemias where alternative splicing occurs as the predominant or only mode of processing of mutant globin transcripts in heterologous cells, alternatively spliced RNAs are detectable at comparatively low levels *in vivo*, probably due to their instability in erythroid cells[6,8,13,14]. Therefore, the apparent absence of such a species from steady-state β^E-bone marrow RNA does not necessarily signify a different processing pattern in erythroid cells, but does prevent full assessment of the contribution of abnormal processing to underproduction of β^E-mRNA. Previously, cytoplasmic instability of β^E-mRNA in erythroid cells has been proposed as another basis for the thalassaemic phenotype[15]. We have observed variable and extensive fragmentation of β^E-mRNA isolated from reticulocytes, but not bone marrow cells, that is consistent with these previous data. However, as most globin is produced in marrow rather than peripheral cells, the contribution of peripheral cytoplasmic instability of β^E-mRNA to decreased β^E-globin synthesis is also unclear. The difficulties in reconciling the results of the surrogate genetic experiments in HeLa cells with those in erythroid cells may largely reflect the rather mild deficit in expression of the β^E-globin gene.

The deleterious effect of a nucleotide substitution in an exon on the rate as well as the pattern of processing of a globin RNA precursor is a newly recognized mechanism of gene dysfunction. The cryptic splice site enhanced by the β^E mutation is also mutated in two other globin genes associated with a thalassaemic phenotype. First, in a β^+-thalassaemic gene, a silent T→A substitution in codon 24 has recently been discovered and shown to affect the processing of RNA transcripts in heterologous cells (A. Nienhuis, personal communication). Second, the predicted DNA sequence GTGGTGAGT in exon-1 of a newly described and underproduced β-chain variant, Knossos ($\beta^{27\mathrm{Ala}\to\mathrm{Ser}}$) (D. Loukopoulos, personal communication) seems as favourable as an authentic donor splice site. We anticipate that transcripts of this β-chain variant will also be alternatively spliced at this cryptic site. Taken together, the underproduction of β-globin from three different genes having mutations within the same cryptic splice site strongly suggests that the alternative RNA splicing we have described is of physiological significance. These principles may easily be expanded to account for underproduction of many gene products associated with inherited disease in man.

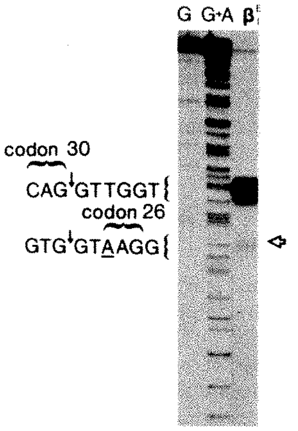

Fig. 2 Location of normal and alternative splices in the β^E gene sequence. Cytoplasmic RNA of HeLa cells transfected with the β^E_1 gene was analysed by S_1 nuclease mapping with the 3'-labelled *Hin*fI probe. G and G+A sequencing reactions[5] of the probe were electrophoresed in parallel lanes. The corresponding sequence of the mRNA strand is shown on the left with the splicing sites indicated by the GT rule[11]. The underlined A in codon 26 is the mutated nucleotide in the β^E-mRNA.

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The deletion in a type of δ^0-β^0-thalassaemia begins in an inverted *Alu*I repeat

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In hereditary persistence of fetal haemoglobin (HPFH) and δ^0-β^0-thalassaemia, increased levels of fetal haemoglobin (HbF) are found in adult individuals. HbF production is particularly noticeable in the former condition in which HbF levels of 20–40% are found in heterozygous patients, as opposed to 5–20% in δ^0-β^0-thalassaemia[1]. In a minority of these cases, no obvious abnormalities have been found in the globin gene region by DNA mapping[2–6], indicating that small deletions or perhaps even point mutations in critical DNA sequences in the globin gene cluster may be responsible for these conditions. However, most cases of HPFH and δ^0-β^0-thalassaemia are associated with extensive deletions in the globin gene cluster[2–4,7–14]. Genetic data[15] and gene mapping investigations[2,4,10] provided some evidence for the location of a regulatory area, whose deletion results in continuing activity of γ-globin genes in adults, in a DNA region between the $^A\gamma$- and δ-globin genes, possibly 3–4 kilobases (kb) 5′ to the δ gene. The precise nature of these sequences is of great interest, because it might help elucidate the molecular mechanisms regulating globin gene expression during development. Recently, Jagadeeswaran *et al.* cloned[16] the DNA encompassing the region of a gene deletion in a type of HPFH[2,10] and showed[16] that the 5′ end point of the deletion lies in the middle of an *Alu*I repetitive DNA sequence[17,18]. We have now cloned the corresponding region from the DNA of a δ^0-β^0-thalassaemia patient[4] and we report here that the deletion ends in a different *Alu*I sequence, ≃700 nucleotides 3′ to the *Alu*I repeat involved in the HPFH deletion, and in the opposite orientation.

The isolation and characterization of the δ^0-β^0-thalassaemic DNA clone will be described elsewhere (B.G. and S.O., in preparation). Figure 1 shows a restriction endonuclease map of the human non-α-globin gene cluster in the region of the $\delta^0\beta^0$ deletion end point. This occurs ~3.8 kb 5′ to the δ-globin gene. Previous analyses[19,20] demonstrated the existence in this area of a pair of repetitive DNA sequences, belonging to the *Alu*I family, pointing in opposite directions and separated by ≃700 base pairs (bp) of non-repetitive DNA.

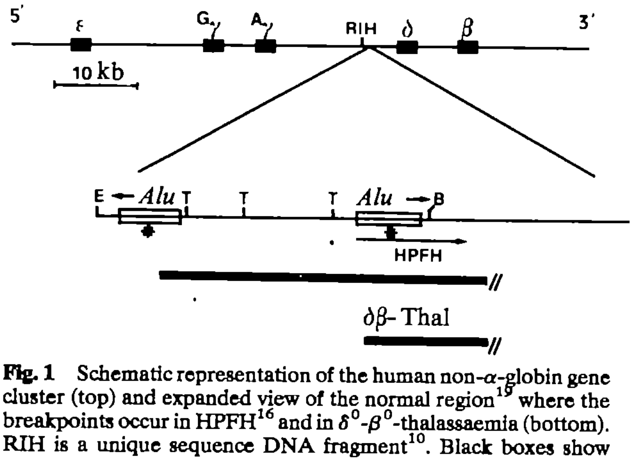

Fig. 1 Schematic representation of the human non-α-globin gene cluster (top) and expanded view of the normal region[19] where the breakpoints occur in HPFH[16] and in δ^0-β^0-thalassaemia (bottom). RIH is a unique sequence DNA fragment[10]. Black boxes show the deletions, short arrows indicate the polarity of the *Alu*I repeats, and the long arrow the transcriptional unit[20]. Asterisks mark the position of sequences homologous to origins of replication of mammalian viruses[17]. E, *Eco*RI site; T, *Taq*I site; B, *Bgl*II site.

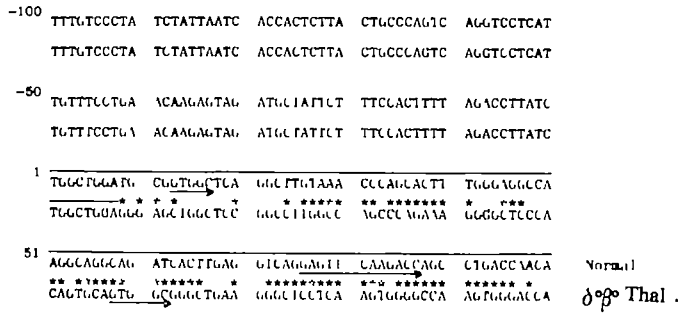

Fig. 2 Comparison of the normal DNA sequence (upper line) with that of DNA across the 5′ end point of the deletion in δ^0-β^0-thalassaemia (lower line). The normal *Alu*I sequence (from nucleotide 1 onwards) is from ref. 20. Asterisks show where the nucleotides of the two sequences differ. Solid lines overlie nucleotides corresponding to *Alu*I DNA. Arrows underlie consensus sequences, possibly involved in specifying transcriptional initiation sites[20,21].

Fine restriction mapping of our clone (S.O. and B.G., in preparation) demonstrates normal bands from the *Eco*RI site to the most 3′ *Taq*I site; we therefore sequenced the δ^0-β^0-thalassaemic DNA 3′ to this *Taq*I site and compared it with the normal DNA sequence (Fig. 2). The two sequences show perfect homology in a 100-nucleotide long fragment. This is followed by an eight-nucleotide sequence in δ^0-β^0-thalassaemic DNA that is identical to the beginning of the normal 3′ *Alu*I repeat; beyond this point the δ^0-β^0-thalassaemic DNA continues into a G-rich region having little homology with the normal *Alu*I sequence. Thus, the $\delta^0\beta^0$ deletion almost completely removes the 3′ *Alu*I sequence, leaving intact the 5′ repeat and non-repetitive DNA in between. In contrast, in the HPFH type sequenced by Jagadeeswaran *et al.*[16], the entire 3′ and part of the 5′ *Alu*I sequence are absent.

HPFH heterozygotes produce during adult life considerably more HbF than δ^0-β^0-thalassaemic heterozygotes. According to the model proposed by Huisman *et al.*[15] (see also refs 2, 4, 10), deletion of an unidentified regulatory sequence between the $^A\gamma$- and δ-globin genes is sufficient to cause a high level of persistent activity of γ-globin genes *cis* to the defect. Thus, comparison of the different deletion 5′ end points in HPFH[16] and δ^0-β^0-thalassaemia identifies the 5′ *Alu*I repeat and/or non-repetitive DNA connecting it to the 3′ *Alu*I repeat as possible candidates for such a key regulatory role. This region is absent from all deletion-type HPFH reported so far, but is present in δ^0-β^0-thalassaemias. (The type of δ^0-β^0-thalassaemia in which expression of the $^G\gamma$ gene exclusively is found[10], is the only apparent exception to this rule. Here, the deletion removes both *Alu*I repeats, but also extends to the $^A\gamma$ gene, thus limiting HbF synthesis; it is probably better considered as a γ-δ-β-thalassaemia.) Although confirmation of the possible regulatory role of this region will require the study of additional HPFH and δ-β-thalassaemias, it is interesting that this region includes sequences for which many different functions have been postulated[17,18]. Here, we only mention that the 3′ *Alu* repeat is transcribed '*in vitro*' by DNA polymerase III[20]. Although the presumed starting site of transcription[20] (position 1 in Fig. 2) is intact, the deletion in δ^0-β^0-thalassaemia removes the internal 'control' region thought to be essential for transcription[20,21]. As nothing is known of their '*in vivo*' transcription, it is premature to speculate about possible roles of the transcription of these sequences in normal and pathological expression of globin genes; however, note that the almost total loss of the 3′ *Alu* repeat in this δ^0-β^0-thalassaemia only slightly changes the phenotype relative to other δ-β^0-thalassaemias in which this region is intact[4]. Finally, attention should also be given to possible effects of distant sequences 3′ to the β-globin gene which are brought by the deletion into direct contact with the *Alu*I repeats; these sequences, which differ between δ^0-β^0-thalassaemic and HPFH DNA (Fig. 2 and ref. 16) are presently

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being located (in collaboration with R. Flavell) by mapping studies and their structure is being analysed.

The mechanism generating the deletion is unknown, but some clue to its understanding may be provided by the sequence of the δ^0-β^0-thalassaemia clone around the breakpoint. In normal DNA the octamer, TGGCTGGA, at the beginning of the *Alu*I sequence is followed, after a five-nucleotide spacer, by a dodecamer, TGGCTCAGGCTT, including a modified version of the former sequence. In δ^0-β^0-thalassaemic DNA, the octamer is intact and is followed, after a six-nucleotide spacer obviously different from the normal one, by a dodecamer which differs at only two positions from the normal one. The normal sequence, by misaligning with a slightly different distant sequence, might thus act as a 'hotspot' for non-homologous recombination, leading to the large deletion observed. Such a mechanism was recently demonstrated in a prokaryotic system[22]. Cloning of the normal counterpart of the distant sequence should allow us to confirm this mechanism for our case.

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Detection of human papillomavirus DNA in anogenital neoplasias

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The presence of papillomaviruses in epithelial-derived cancers from several animal species[1–3] has led to the speculation that these viruses may also have a pathogenic role in the development of certain human carcinomas, particularly those associated with the anogenital tract[4]. Recently, human papillomavirus (HPV) DNA has been detected in epithelial-derived cancers, both cutaneous[1,5] and metastatic[5], from patients exhibiting the rare, chronic flat wart disease, epidermodysplasia verruciformis (EV). Except for patients exhibiting this chronic wart syndrome, the association of HPV genomes with human epithelial cancers has not been demonstrated[6]. In an attempt to delineate the association and possible involvement of papillomaviruses with human anogenital carcinomas, we have begun an analysis of these cancers for the presence of HPV-specific nucleotide sequences by using highly sensitive hybridization procedures capable of detecting distantly related papillomaviruses at low copy number. Here we demonstrate the presence of HPV DNA in several types of anogenital tumours: Bowenoid papulosis, carcinoma *in situ*, and verrucous carcinoma. These data indicate that HPV can be detected in several types of premalignant and malignant tumours, supporting the contention that this group of viruses may be involved in the development of certain types of human epithelial-derived cancers.

We have used the Southern blot hybridization procedure to search for HPV DNA in three types of anogenital tumours verrucous carcinoma, which is a form of invasive squamous cell carcinoma; carcinoma *in situ*; and Bowenoid papulosis, which is histologically identical to but biologically distinct from carcinoma *in situ*. Total cellular DNA was extracted from tumour tissue as described previously[5] and analysed by the Southern blot hybridization technique[7]. The HPV DNA probes used in these analyses included HPV-EV and HPV-5 obtained from patients with epidermodysplasia verruciformis[5,8], and HPV-6 obtained from a patient with condylomata accuminata, a form of anogenital wart[9]. The HPV DNAs were molecularly cloned into pBR322 and the recombinant plasmids were labelled with ^{32}P *in vitro* by nick-translation[10] resulting in specific activities of 5×10^8 c.p.m. μg^{-1}. Because of the apparent lack of homology between different types of HPV in stringent hybridization[11,12], conditions of reduced stringency were used in these analyses. In such low stringency conditions, any specific type of HPV will hybridize to any of the other known varieties of

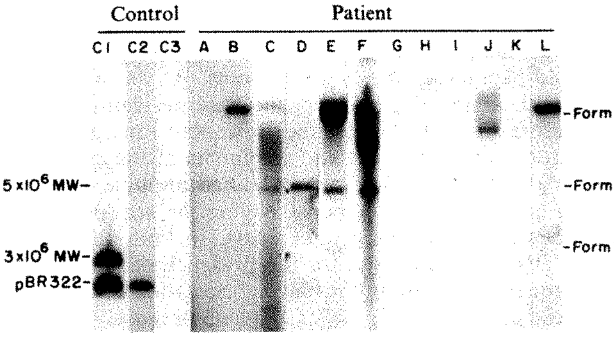

Fig. 1 Detection of HPV DNA in tumour tissue. Total cellular DNA was extracted from tumour tissue as previously described[5]. The DNA was electrophoresed through 1% agarose gels and transferred to nitrocellulose filters by the method of Southern[7]. The filters were dried at 80 °C under vacuum for 2 h and hybridized in conditions of low stringency. The HPV used as probe in these conditions was an HPV species that exhibits homology (~36%) with HPV type 3 in stringent conditions (ref. 8 and M. Green, personal communication) and which for brevity is referred to here as HPV-EV. The filters were pre-hybridized at 37 °C for 24 h in hybridization solution containing 500 μg ml^{-1} depurinated salmon sperm DNA, 0.1% each of bovine serum albumin, Ficoll 400 and polyvinylpyrrolidone, 20 mM sodium phosphate *p*H 6.8, 1 M sodium chloride, 0.1% SDS, and 30% formamide[11]. Fresh hybridization solution containing nick-translated ^{32}P-HPV-EV probe (containing both cloned *Bam*HI fragments (5 ng probe per ml solution; 2.5×10^6 c.p.m. ml^{-1} and 10% dextran sulphate) was added to the filters and incubated at 37 °C for 20 h. Filters were washed three times in 2×SSC *p*H 7.0 (0.3 M sodium chloride, 0.03 M sodium citrate), 0.1% SDS, and 0.1% Na pyrophosphate at room temperature and for an additional 4 h in 3× SSC *p*H 7.0, 0.1% SDS, and 0.1% Na pyrophosphate at 40 °C. Filters were dried and autoradiographed at −70 °C with an intensifying screen. Lane C1 contains 10 pg of the 3.0-megadalton fragment of the HPV-EV DNA used as probe that had been cleaved from the pBR322 plasmid with *Bam*HI; also present is the remaining 10 pg of pBR322. Lane C2 contains 100 pg of linear HPV-5 that was prepared from a plasmid composed of HPV-5 and pBR322. The plasmid was cleaved with *Bam*HI and the fragments separated by agarose gel electrophoresis. The linear HPV-5 band was cut out of the gel and the DNA was electroeluted from the agarose as previously described[5]. The lower band is a trace amount of pBR322 that remained in the preparation. Lane C3 contains 10 μg of cellular DNA from a hyperkeratotic lesion. Lanes A–L contain 5–10 μg of cellular DNA from the corresponding patient. The DNA in lane D was cleaved with *Bam*HI, while the remaining cellular DNA samples were not treated with restriction endonucleases.

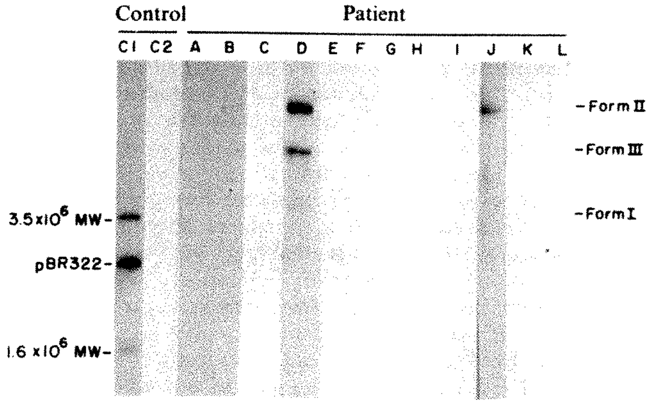

Fig. 2 Hybridization of DNA from tumour tissue with ^{32}P-HPV-6 in stringent conditions. DNA was prepared, electrophoresed and transferred to nitrocellulose as described in Fig. 1 legend. The stringent hybridization conditions were similar to those described in Fig. 1 with the following differences: (1) the pre-hybridization and hybridization solutions contained 50% formamide; (2) the hybridization was carried out at 40 °C; (3) after the room temperature washes, the filters were washed for an additional 4 h in 0.4× SCC *p*H 7.0, 0.1% SDS, 0.1% Na pyrophosphate at 50 °C; and (4) the probe used was nick-translated pBR322-HPV-6. Lane Cl contains 10 pg of the 3.5-megadalton fragment and 10 pg of the 1.6-megadalton fragment of HPV-6 DNA; each has been cleaved from the pBR322 plasmid with a *Bam*HI and *Eco*RI double digestion. Lane C2 contains 10 μg of cellular DNA from a hyperkeratotic lesion. Lanes A–L contain 5–10 μg of cellular DNA from the corresponding patient.

HPV by virtue of distantly conserved nucleotide sequences[12,13]; using these conditions, no hybridization can be detected between DNA from any HPV type and simian virus 40 or BK DNA[13]. The sensitivity of our low stringency hybridization procedure allowed detection of as few as six copies of heterologous HPV genome equivalents per diploid cell (see Fig. 1, lane C2).

Tumour samples were analysed from two patients with Bowenoid papulosis. One of the patients (A) was a 35-yr-old female with a single tumour on the external genitalia. Four years before the detection of the Bowenoid tumour she was treated for anogenital warts and carcinoma of the cervix. The second patient (B), a 33-yr-old male, had multiple Bowenoid tumours on the shaft of the penis. Neither he nor his wife had a previous history of anogenital warts. In both cases, when cellular DNA was analysed for the presence of HPV-related DNA sequences by Southern blot hybridization in conditions of reduced stringency (T_m −40 °C), we observed HPV-specific nucleotide sequences migrating in an identical manner to unintegrated forms of HPV DNA (Fig. 1).

The verrucous carcinoma samples were obtained from a 61-yr-old female (C) with a history of squamous cell carcinoma of the cervix and a verrucous carcinoma on the perineum, and from a 17-yr-old male (D) with a penile verrucous carcinoma. These tissue samples showed unintegrated HPV DNA in less stringent hybridization conditions (Fig. 1).

Eight vulvar carcinoma *in situ* tissue samples were also obtained for analysis. Four out of eight DNA samples from these patients contained HPV-related DNA when analysed by hybridization in less stringent conditions (see Fig. 1, patients E–L).

Due to the association of HPV-5 DNA with carcinomas from patients exhibiting epidermodysplasia verruciformis[1,5], we also tested DNA extracts from many of the verrucous carcinomas and carcinomas *in situ* with labelled HPV-5 in stringent conditions where we could detect as little as 0.1 viral genome copy per cell genome equivalent. With these conditions we were unable to detect any HPV-5-related sequences (Table 1). Moreover, we have tested these tumour DNAs for nucleotide sequences homologous to an HPV (type 6) that is associated with anogenital papillomas[14] and find most of these tumours negative for HPV-6-related sequences (Fig. 2, Table 1).

The viral DNA extracted from each patient's tissue was found to be of typical HPV genome size (~5 megadaltons) with no evidence of any subgenomic fragments, as has been shown in carcinomas from patients with epidermodysplasia verruciformis[5]. Based on the electrophoretic mobility of form I (covalently closed circular), form II (nicked circular) and form III (linear) viral DNA, most of the viral DNA obtained from these tissues seems to be unintegrated although we cannot completely exclude the possibility of integrated viral sequences using this detection technique. Sufficient HPV DNA was not available to perform restriction endonuclease mapping to type the HPV species in these tumours.

From the Southern blot hybridization analysis presented here, it is clear that HPV-specific nucleotide sequences are present in several different anogenital tumour samples. Our ability to detect as few as six copies of heterologous HPV genome equivalents per cell genome using conditions that facilitate hybridization with heterologous HPV types enhanced our ability to detect HPV sequences. This technique is thus more sensitive than that used in a recent study in which carcinomas were analysed for the presence of HPV DNA in stringent hybridization conditions which only detected HPV DNA closely related to that of the radiolabelled HPV DNA probe[6]. Moreover, we have detected HPV DNA in anogenital cancers that were previously thought to lack HPV[14]. At present it is unclear what, if any, is the specific involvement of HPV in the development of these anogenital tumours. The 12 patients analysed in this study exhibited three clinical syndromes each characterized by either benign, premalignant or malignant transformation of anogenital skin. The presence of HPV DNA in these tumours suggests but does not implicate a pathogenic

Table 1 Summary of lesions examined and detection of HPV-related sequences

Lesion	Patient	Age	Site	Less stringent (^{32}P-HPV-EV)	Stringent ^{32}P-HPV-5	^{32}P-HPV-6
Bowenoid papulosis	A	35	Vulva	+	ND	–
	B	33	Penis	+	ND	+
Verrucous carcinoma	C	61	Vulva	+	–	–
	D	17	Penis	+	–	+
Carcinoma *in situ*	E	20	Vulva	+	–	–
	F	40	Vulva	+	–	*
	G	27	Vulva	–	–	–
	H	44	Vulva	–	–	–
	I	42	Vulva	–	ND	–
	J	77	Vulva	+	ND	+
	K	32	Vulva	+	–	–
	L	84	Vulva	–	ND	–

+, HPV sequences detected; –, HPV sequences not detected; ND, not determined.

* When the small amount of viral DNA seen in stringent conditions (Fig. 2, patient F) is compared with the large amount seen in less stringent conditions (Fig. 1, patient F), there are two possible explanations: either there is more than one type of HPV present in this tumour and HPV-6 represents a very low percentage of the total viral DNA or there is such a high concentration of viral DNA in the sample that low level cross-hybridization has occurred. As neither possibility can be conclusively excluded by the data presented, we prefer not to classify the HPV type present.

role for papillomaviruses. Since several patients had a previous history of wart disease it is possible that latent virus or viral DNA remained in the skin of these patients after eradication of the wart disease. In support of this contention is the identification of HPV DNA in normal skin of some patients with EV disease (R.S.O. *et al.*, in preparation). On the other hand, the history of previous wart disease was not a criterion for choosing the patients. Our original interest was in patients with multifocal tumours which clinically resembled warts. These studies, therefore, also include patients that exhibited no previous history of wart disease, and we similarly found HPV DNA associated with their tumours. It is conceivable that either these patients may have had small lesions that were undetected or they were infected subclinically. Nevertheless, our studies indicate that the entire spectrum of anogenital tumours, from benign to malignant, can carry free HPV DNA genomes. More patients must be studied to understand the prevalence of this association. Restriction endonuclease mapping and other molecular studies must be performed to characterize the virus type. As many patients with anogenital wart disease do not develop genital malignancies, other factors may be involved. Host immunity, co-carcinogens and infection with other virus groups may also have a pathogenic role with HPV in the process of malignant conversion.

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Note added in proof: After submission of this manuscript, Green *et al.* reported the presence of HPV-specific DNA in several different anogenital carcinomas[8].

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Localization of the normal allele of T24 human bladder carcinoma oncogene to chromosome 11

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The development of DNA-mediated gene transfer techniques[1,2] has made it possible to identify transforming genes present in certain human tumour cells. Such genes have been shown to induce morphological transformation when used to transfect suitable assay cells[3–10]. Recently a transforming gene has been isolated by molecular cloning techniques from the T24 (ref. 11) and EJ (ref. 12) human bladder carcinoma cell lines[10,13,14]. This bladder carcinoma oncogene has been shown to be of human origin, less than six kilobase pairs (kbp) in size, and closely related to the *onc* genes (v-*bas* and v-*ras*) of BALB and Harvey murine sarcoma viruses[15–17]. These transforming retroviruses arose in nature by transduction of cellular genes from mouse and rat cells, respectively[18]. To understand better the relationship of the T24 oncogene with other human cellular genes, we have determined the chromosomal location of its normal allele within the human genome. We show here that it is carried on chromosome 11 in normal cells.

Somatic cell hybrids were constructed between various human cell lines and either mouse or Chinese hamster cells. Such hybrid cells segregate human chromosomes and will only show the presence of the corresponding human gene when the relevant chromosome has been retained. Thus, comparison of the presence or absence of the human gene with the known karyotype of a number of somatic cell hybrids allows assignment of the gene to a particular human chromosome.

To map the normal allele (c-*bas*) of the T24 oncogene, a 3.0-kbp *Sac*I subfragment containing v-*bas* related sequences[17] was used as a probe. As shown in Fig. 1, this probe detected a 20-kbp fragment in *Eco*RI-cleaved human placenta DNA. Using stringent hybridization conditions, hybridization with related genes present in either mouse or Chinese hamster DNA was not found. The 20-kbp fragment was observed in 4 of 18 somatic cell hybrids (Fig. 1) in one series (Fig. 2) examined. Analysis of two additional series of hybrid cell lines (*b* and *d*, Table 1) showed that hybridization to the T24 probe correlated only with the presence of human chromosome 11.

To confirm assignment of the normal allele of the T24 oncogene to chromosome 11, six hybrid cell lines were subcloned, including three lines which contained chromosome 11. This procedure allowed further human chromosome segregation. Spontaneous loss of chromosome 11 was observed in many subclones (14/32) of the three parental lines originally retaining this chromosome. However, detection of T24 oncogene sequences by molecular hybridization in each series of subclones correlated with the presence of chromosome 11 (Table 1*c*) and therefore the normal human homologue of the T24 oncogene can be unambiguously assigned to this chromosome.

Previously assigned markers carried on human chromosome 11 include lactate dehydrogenase A (LDH-A) and esterase-A4 (EsA4) which have been regionally localized to the short arm (11p1203–11p1208) and proximal portion of the long arm (11cen–11q22) respectively[19]. The presence of the normal allele of the T24 oncogene correlated with expression of human LDH-A in all hybrids. However, human EsA4 was detected in four additional hybrid lines (Table 1*b*) which did not contain this oncogene or human LDH-A. The chromosome 11 break point in these four lines has not been determined by karyotypic analysis, but a fragile site has been previously described on the proximal portion of the long arm (11q13) of chromosome 11 in cultured cells[20]. Our results currently exclude the distal portion of the chromosome 11 long arm as the locus of the T24 transforming gene.

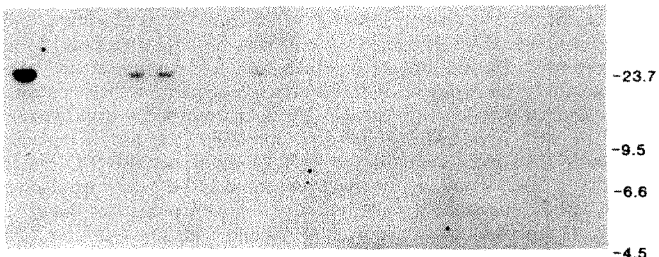

Fig. 1 Hybridization of the normal allele of the T24 oncogene to *Eco*RI-digested DNA from human–Chinese hamster somatic cell hybrids. Human and Chinese hamster control DNAs were run in lanes 1 and 2 respectively. Lanes 3–20 contained DNAs from 18 somatic cell hybrids of series *a* (Table 1). After size-fractionation by electrophoresis, DNAs were transferred to nitrocellulose filters and hybridized to a nick-translated 3-kbp cloned insert derived from the normal allele of the T24 oncogene[17]. The positions of *Hin*dIII-digested λ DNA fragments are indicated on the right.

Normal cellular alleles of the T24 oncogene can be transforming genes in a variety of conditions. The transduction of mouse or rat cell homologues of this gene by retroviruses had led to the creation of BALB- and Harvey-murine sarcoma viruses. Recent studies have shown that the normal human gene itself can be manipulated *in vitro* by the addition of retroviral long terminal repeat sequences so as to acquire transforming activity[21]. Only minor genetic alterations lead to activation of this gene as a transforming gene in T24 bladder carcinoma cells. By restriction mapping and heteroduplex analysis, the normal gene could not be distinguished from the T24 oncogene in the region required for transforming activity[17], excluding any major insertion, deletion or major rearrangement of sequences. In fact, recent findings indicate that a point mutation affecting the twelfth amino acid residue from the amino terminus of the T24 oncogene product accounts for its transforming activity[22,23].

The potential importance of *onc* genes of transforming retroviruses for understanding basic mechanisms involved in malignant transformation, has recently led to the chromosomal mapping of several of these genes. Thus, the human homologue of v-*sis*, the *onc* gene of simian sarcoma virus, has been assigned to chromosome 22 (refs 24, 25) while *mos* (ref. 26), *fes* (refs 27, 28), *myb* (ref. 27), and *abl* (ref. 28) have been mapped to chromosome 8, 15, 6 and 9, respectively. Our chromosomal assignment of the normal allele of the T24 ocncogene to chromosome 11 represents the first mapping of a cellular gene shown to be activated as a transforming gene in a human tumour. Knowledge of the locations of this and other *onc*-related genes has obvious importance for determining the relationship, if any, between known chromosomal aberrations in naturally occurring tumours and oncogenes found on such chromosomes.

Table 1 Segregation of the normal allele of the T24 oncogene with specific human chromosomes

Series	Total hybrids	T-24 allele positive	% Discordancy	
			Chromosome 11	Other chromosomes
a	18	4	0	≥11
b	19	3	0	≥16
c	56	18	0	≥16
d	22	10	0	≥23

Assignment of the normal allele of the T24 oncogene to human chromosome 11 is based on analysis of the segregation of this gene with specific human chromosomes in human–rodent somatic cell hybrids. Cell pellets were simultaneously prepared for DNA isolation and isoenzyme analyses from three series of somatic cell hybrids (*a*, *b*, *d*) and subclones of six hybrid cell lines (*c*). The 3.0-kbp *Sac*I probe was hybridized to nitrocellulose filters containing *Eco*RI-digested, size-fractionated cellular DNAs. The human chromosomes present in each hybrid cell line were determined from starch gel electrophoretic analyses[31,32] of isoenzyme markers that have been previously assigned to each of the human chromosomes[19] as described[24]. Preparation and chromosomal analysis of hybrid series *a* (ref. 33) and *d* (ref. 24) have been reported. Hybrid series *b* is similar to *a*, and it represents hybrid lines isolated after fusing well-characterized human fibroblasts containing a balanced translocation between the X cxhromosome and chromosome 14 with *hprt*$^-$Chinese hamster fibroblast[33]. *c* Series denotes subclones of six hybrid cell lines from *d*. Concordancy refers to either retention or loss of both a human chromosome and the gene from each line, whereas independent segregation of a chromosome and the gene represents discordancy. Detection of the normal allele of the T24 oncogene could be correlated with the presence or absence of human chromosome 11 in all hybrid cell lines. The minimum discordancy between segregation of all other human chromosomes and the oncogene is shown with the following exceptions: *a*, 0% and 6% discordancy with chromosomes 13 and 16, respectively; *b*, 0% and 11% discordancies with chromosomes 3 and 4 and chromosome 10, respectively; *c*, 5, 7, 9 and 9% discordancies with chromosomes 22, 1, 6 and 9, respectively. The long arm of chromosome 11 alone (EsA4 marker) did not segregate concordantly with the T24 oncogene in series *b* (see text).

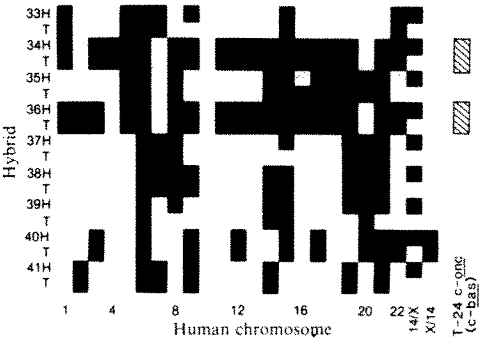

Fig. 2 Distribution of specific human chromosomes in human–hamster cell lines of *a* (Table 1). Hybrids were isolated after fusing well characterized human fibroblasts (GM0073) containing a reciprocal X;14 translocation with *hprt*$^-$ Chinese hamster fibroblasts and cloning each independent line twice[33]. Individual hybrid cell lines are represented on the ordinate and H or T indicates expansion of that cell line in HAT (100 μM hypoxanthine, 1 μM amethopterin and 16 μM thymidine) or 50 μM 6-thioguanine, respectively. Specific human chromosomes are represented on the abscissa. Solid boxes indicate the presence of a particular human chromosome in a hybrid line, and open boxes indicate absence of the chromosome. The presence of the normal allele of the T24 oncogene is shown by hatched boxes in the last column.

The relevance of human chromosome 11 to cancer-associated genes has previously been restricted to the association of a deletion on this chromosome with Wilms' tumour, a malignant renal tumour of children[29]. The deletion has been localized to 11p13 by binding techniques[30]. This deletion could lead to loss of some normal function or to a rearrangement that might result in gene activation. Although we cannot yet precisely localize the T24 oncogene to this specific region, it is tempting to speculate that an association may be involved since the oncogene segregates concordantly with a short arm (11p) marker and discordantly with a long arm (11q) marker for this chromosome.

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Low Ca^{2+} impedes cross-bridge detachment in chemically skinned *Taenia coli*

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Muscle force is generated by cycling cross-bridges between actin and myosin filaments. In smooth muscle, cyclic attachment and detachment of cross-bridges is thought to be induced by a Ca^{2+}- and calmodulin-dependent myosin light chain kinase which phosphorylates myosin. The relaxation that occurs after Ca^{2+} removal is usually ascribed to dephosphorylation of myosin by a phosphatase as non-phosphorylated myosin is unable to form force-generating criss-bridges[1]. Recently, Dillon *et al.*[2] claimed, however, that dephosphorylation of attached cross-bridges may impede cross-bridge detachment, thus forming so-called 'latch bridges'. Here we present evidence that after a Ca^{2+}- and calmodulin-induced contraction of chemically skinned guinea pig *Taenia coli*[3], the rapid removal of Ca^{2+} impedes the detachment of the myosin cross-bridges from the actin filament; force can then be maintained without energy consumption. The extremely slowly detaching cross-bridges which maintain the force after Ca^{2+} removal may indeed correspond to the 'latch bridges' mentioned above.

The Ca^{2+}-jump technique[4] allows the Ca^{2+} concentration to be changed in small bundles of chemically skinned muscle fibres within fractions of a second. Compared with this time scale, the mechanical response in chemically skinned *T. coli* is much slower; the broken line marked ±Ca^{2+} in Fig. 1 shows the time at which the Ca^{2+} concentration was changed from $<10^{-8}$ M to 10^{-5} M and vice versa. The force generated by the fibre (solid line) shows a marked delay in response to Ca^{2+}. It is conceivable that this delayed relaxation was due to an internal viscoelasticity independent of the actin–myosin interaction. Such a 'passive' viscosity would probably not be affected by inorganic phosphate. However, the relaxation was markedly accelerated after addition of 6 mM inorganic phosphate to the incubation solution.

Figure 1 shows that in contrast to the force (solid line), the ATPase activity (open circles) decreased immediately after Ca^{2+} removal. Thus, the high force maintained after Ca^{2+} removal must be ascribed to non-cycling but attached and force-maintaining cross-bridges. The simplest interpretation of our findings is that the rate constant of the cross-bridge detachment is similar to the rate constant of tension decrease, that is, $\sim 0.1\ \mathrm{min}^{-1}$. From the ATPase activity (Fig. 1) and the total myosin concentration in this type of muscle[5], the turnover rate for the actin–myosin interaction was estimated to be $\sim 0.25\ \mathrm{s}^{-1}$ at 10^{-5} M Ca^{2+}, assuming that all myosin molecules are interacting with actin and that they interact with only one head at the same time. As ATP splitting requires a complete cross-bridge cycle (including detachment and reattachment of the cross-bridge), the turnover time of the cross-bridge cycle must be longer than the time needed for cross-bridge detachment. Thus the detachment rate of the cross-bridges after Ca^{2+} removal (estimated

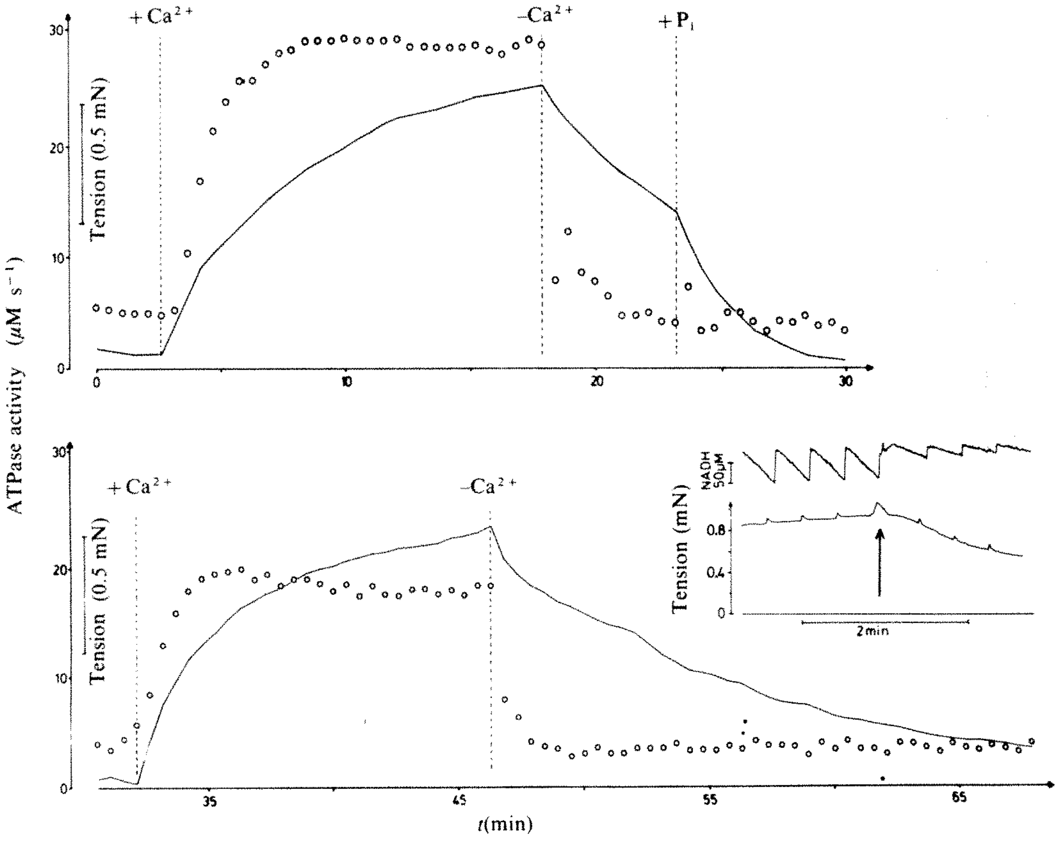

Fig. 1 Force and ATPase activity of chemically skinned *T. coli* from guinea pig. The solid line shows the generated force, the open circles the simultaneously measured ATPase activity (mol ADP l^{-1}, fibre vol s^{-1}). The dashed line marks the time at which the Ca^{2+} concentration was changed from $<10^{-8}$ M to 10^{-5} M (+Ca^{2+}) and vice versa (−Ca^{2+}). The time at which 6 mM phosphate was added to the incubation solution is indicated by the broken line marked P$_i$. The 'tension cost' has a maximum at the start of the contraction (see ref. 9). ATPase activity was measured using an NADH-coupled optical assay method; in this assay the consumption of NADH corresponds to ADP production. A fibre bundle (0.3 mm diameter, length 7 mm) was mounted in a narrow chamber (diameter 0.8 mm) which was perfused with an ATP–salt solution (pH 6.7, $T = 20$ °C) consisting of: 30 mM imidazole, 7.5 mM ATP, 10 mM MgCl$_2$, 1 mM NaN$_3$, 1 mM phosphoenolpyruvate, 4 mM EGTA, CaEGTA, 100 U ml^{-1} pyruvate kinase, 140 U ml^{-1} lactate dehydrogenase, 0.6 mM NADH, 0.2 mM P^1,P^5-di(adenosine-5'-)pentaphosphate, 2 mM dithioerythritol, 0.1 μM calmodulin (for the effect of calmodulin see ref. 3). To measure the NADH consumption, the perfusion was stopped for 30 s (see inset: upper trace, NADH concentration in the chamber; lower trace, force. The time at which the Ca^{2+} concentration was changed from 10^{-5} M to $<10^{-8}$ M is marked by an arrow). The change in the NADH fluorescence intensity with time in both chamber and fibre was recorded with a Zeiss microscope photometer. After the measurement, the chamber was perfused again with fresh solution, and the procedure was repeated.

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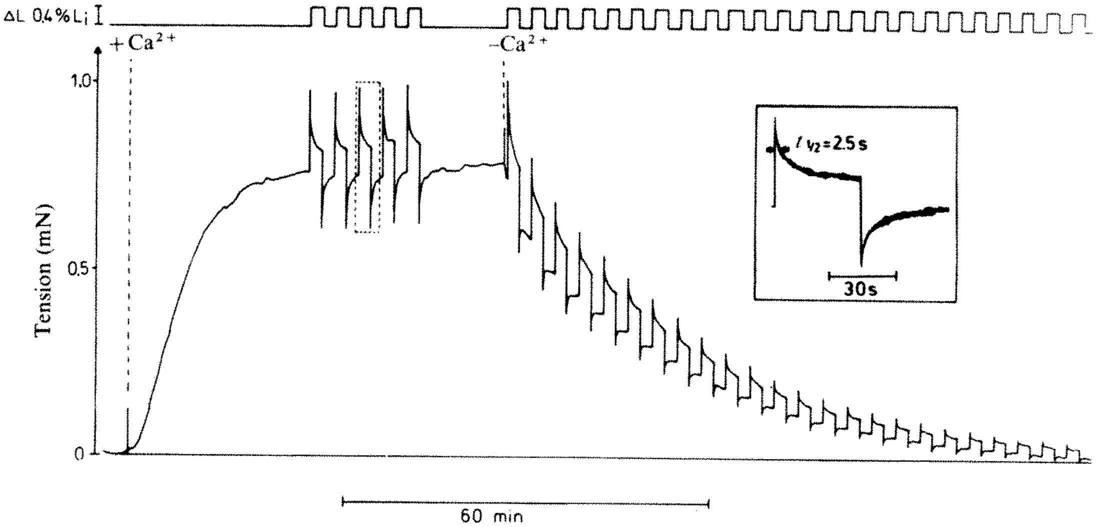

Fig. 2 Force response of a repetitively stretched and released skinned *T. coli*. The upper trace shows the length signal, the lower trace the corresponding force. The broken line marks the time at which the Ca^{2+} concentration was changed from $<10^{-8}$ M to 10^{-5} M (+Ca^{2+}) and vice versa (−Ca^{2+}). The inset shows, with an expanded time scale, the force transient of one stretch–release cycle. Conditions as described in Fig. 1 legend. ΔL, 0.4% L_i = length change as % of the initial length (L_i).

above to be ~0.1 min^{-1}) must be over 100-fold slower than the detachment in the presence of Ca^{2+}. It has been concluded from biochemical studies, however, that ATP may also be split by myosin–S1 being attached to actin[6]. Thus the cross-bridge cycle of attachment and detachment could be much slower than indicated by the rate of the ATP splitting process. To obtain a different estimate for the cross-bridge detachment rate, contracted fibre bundles were stretched and released repetitively (see Fig. 2: upper trace, muscle length; lower trace, the corresponding force). A plausible interpretation of the force transients obtained is that after the length change, the force changes (with a half time ($t_{1/2}$) of ~2.5 s) until the cross-bridges that are attached at the moment of the length change are replaced by others. The kinetics of this process is the same for small stretches and for small releases, indicating that it also reflects the cross-bridge kinetics of the isometric contracting state. If this is so, and if the detachment limits the rate of the force recovery after the length change, then the rate of cross-bridge detachment in the Ca^{2+}-activated state presumably must be greater than ~0.5 s^{-1} (compared with 0.1 min^{-1} after Ca^{2+} removal). The tension recovery following length perturbations after Ca^{2+} removal seems to be slower and less complete (see also Schneider *et al.*[7]). This finding is also consistent with a slow detachment of cross-bridges after Ca^{2+} removal.

The results reported here indicate that after Ca^{2+} removal, cross-bridge detachment occurs much more slowly than in the presence of Ca^{2+}. The absence of Ca^{2+} obviously inhibits not only the myosin cross-bridge attachment to actin but it also impedes cross-bridge detachment from actin (see also 'catch' state in the Byssus retractor[8]). Whether this is a direct effect of the lack of Ca^{2+} or an indirect effect due to dephosphorylation of myosin remains to be determined. In any case, after Ca^{2+} removal, non-cycling cross-bridges or those that are still attached cause the high force to be maintained with very low ATPase activity.

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Orientation of spin labels attached to cross-bridges in contracting muscle fibres

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Electron micrographs showing different cross-bridge orientations in different states of muscle fibres, and X-ray diffraction patterns indicating axial cross-bridge disorder in contracting muscle first suggested that force generation in the contracting muscle involved a change in orientation of the myosin heads that form cross-bridges between thick and thin filaments[1,2]. This has been supported by subsequent work; the myosin molecule has the required flexibility for changes in orientation[3,4]. The orientation of muscle tryptophans and of probes attached to the myosin heads of permeable muscle fibres depends on the state of the muscle[5–9]. Recently, fluorescence polarization fluctuations and time-resolved X-ray diffraction patterns have suggested that cross-bridges of a contracting muscle can rotate[10,11]. We have used electron paramagnetic resonance (EPR) spectroscopy to monitor the orientation of spin labels attached specifically to a reactive sulphydryl on the myosin heads in glycerinated rabbit psoas skeletal muscle. Previously, it has been shown that the paramagnetic probes are highly ordered in rigor muscle, with a nearly random angular distribution in relaxed muscle[12]. We show here that during the generation of isometric tension, ~80% of the probes display a random angular distribution as in relaxed muscle while the remaining 20% are highly oriented at the same angle as found in rigor muscle. These findings indicate that a domain of the myosin head does not change orientation during the power stroke of the contractile interaction.

The maleimide spin label (MSL) can be reacted selectively with a reactive sulphydryl on the myosin head (SH$_1$) in a glycerinated rabbit psoas muscle fibre[12,13]. The EPR spectra can be used to determine directly and unambiguously the angular distribution of the probes relative to the magnetic field[12,13]. Here we have used spin labels attached to SH$_1$ to

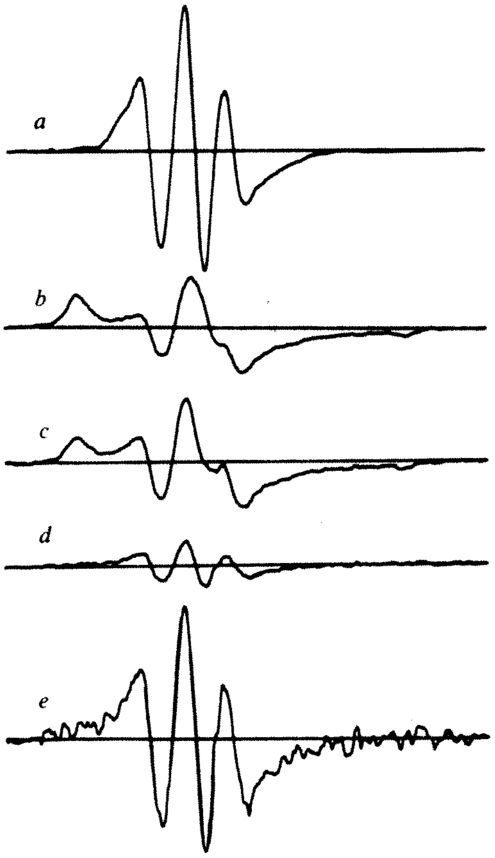

Fig. 1 EPR spectra of spin labels attached to the myosin heads during rigor (*a*), relaxation (*b*) and isometric tension (*c*). Spectrum *d* is a difference spectrum, obtained by subtracting 81% of spectrum *b* from spectrum *c*. This difference spectrum is shown again in *e*, amplified by 5.3 so that it represents the same number of probes as spectra *a–c*. Glycerinated rabbit psoas fibres were labelled with MSL by the procedure of Thomas and Cooke[12] except that the label concentration was increased to 0.5 mM, the *p*H was 6.5, and the duration of labelling was 10 min. Approximately 50–70% of the heads were reacted at the SH_1 as judged by extraction of myosin and measurement of ATPase activity in the presence of EDTA. Spectra were obtained at 24 °C with a Varian E109E spectrometer interfaced to a Northstar computer. The E238 cavity was modified to accept a capillary (1.2 mm inside diameter) parallel to the applied magnetic field. About 100 fibres, in bundles of 5–10, were secured on either end with surgical thread and pulled into the capillary, almost filling it. Solution was allowed to flow past the fibres at 0.3 ml min^{-1} (that is, with a velocity of 2–5 cm s^{-1}), aiding perfusion of the bundles. No differences in the spectra were seen when the bundle size or flow rate was increased or decreased by a factor of 2. Fibres in rigor were bathed in 'rigor buffer' (0.12 M KCl, 5 mM $MgCl_2$, 2 mM EGTA and 20 mM MOPS, *p*H 7.0). Relaxed spectra were obtained by addition of 5 mM ATP, 20 mM CP and 0.4 mg ml^{-1} creatine kinase to the rigor buffer. Contraction was induced by adding 2 mM $CaCl_2$. After contraction the fibres were returned to rigor solution, and data were used only when the reappearance of a well ordered spectrum indicated that sarcomere heterogeneity had not developed during contraction. The baseline for each spectrum is 100 G wide.

investigate cross-bridge orientation during isometric contraction of skeletal muscle.

Figure 1 shows the EPR spectra of MSL-labelled muscle fibres held isometrically in conditions of rigor (Fig. 1*a*), relaxation (*b*) and tension (*c*). The spectrum of fibres in rigor indicates that the probes are highly oriented, with a gaussian angular distribution of 15° (full width at half maximum) centred about an angle of 82 ± 1° with respect to the fibre axis[12]. When ATP is added to relax the fibres, the EPR spectrum shows that the angular distribution of the probes is almost isotropic[12] (Fig. 1*b*). Addition of Ca^{2+} induces tension (see Fig. 1*c*); this spectrum can be shown to be a superposition of the spectra obtained in rigor and relaxation. When 81% of the spectrum in relaxation (Fig. 1*b*) is subtracted from that in tension (Fig. 1*c*), the difference spectrum is identical to that of rigor fibres (Fig. 1*d*, *e*). Subtraction of different fractions (not shown) shows clearly that the correct disordered fraction lies between 75 and 85%. Thus, under isometric tension, 80 ± 5% of the myosin heads have probes disoriented as in relaxed muscle, while the probes on the remaining heads are highly oriented similar to fibres in rigor. The most dramatic aspect of Fig. 1*c* is that no new probe angles, different from those seen in rigor or relaxation, are observed. The resolution of the spectrum is high. If over 5% of the probes assumed a new, well defined angle differing from the rigor angle by >10°, or if over 10% of the probes were less disordered than in relaxation (that is, distributed over <90°) they would have been detected.

A first question which arises from the spectrum of Fig. 1 is whether the 'rigor-like' component of the spectrum obtained during force generation represents a state in the normal cycle of the cross-bridge or whether it is due to some portion of the sample that is in rigor because of an inadequate supply of ATP. We have addressed this question by varying the concentration of ATP or creatine phosphate (CP), and by observing the speed at which small molecules can diffuse into the fibres. Figure 2 shows the fraction of disoriented probes as a function of the ATP concentration. The fraction of disoriented probes approaches 75% as the concentration of the substrate approaches infinity. A similar result was obtained when the concentration of CP was varied. Thus, the myosin heads seem to be saturated with substrate in the conditions used in Fig. 1. The conclusion was further strengthened by several additional experiments. Following a change in the perfusing solution from a rigor solution to a relaxing solution, the spectral changes occurred exponentially with a time constant of 5 s, showing a rapid rate of substrate diffusion into the fibres. If the flow of the perfusing solution was abruptly stopped during contraction, no increase in the rigor component was observed for over 40 s, indicating that the fibres contain sufficient substrate for this

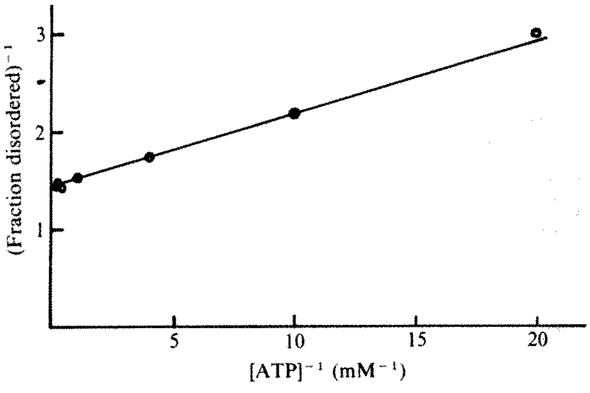

Fig. 2 The fraction of disoriented probes as a function of the ATP concentration over the range 50 μM to 10 mM; conditions are the same as in Fig. 1*c*, except for ATP concentration conditions. The fractions of disoriented and oriented probes are proportional to the heights of the first (P_1) and second (P_2) spectral peaks, respectively. Because a given peak height for P_1 represents twice as many probes as P_2 (see Fig. 1 *a*, *b*), the fraction of disoriented probes is $2P_1/(2P_1+P_2)$. The inverse of this fraction is plotted in Fig. 2, as a function of the inverse of the ATP concentration. If the fraction of oriented probes decreased to zero as substrate increased the data should extrapolate at the ordinate to 1, the value for relaxed fibres. This does not occur, and the extrapolated value shows that ~25% of the probes remain oriented as the substrate concentration approaches infinity.

time period and that the magnitude of the rigor-like component is not due to a balance between the rate of substrate diffusion into the fibres and its consumption.

We conclude that all the myosin heads in our fibres were in the presence of saturating ATP, therefore that the different spectral components corresponded to different states in the force-producing cross-bridge cycle. Thus, in isometric tension, the probed domain in each myosin head spends about 80% of its time in a state with disorientation identical to that of relaxation, and about 20% of its time in a state having an orientation identical to that of rigor. Reaction with the label decreased the active tension of the fibres by ~20%. Further reaction of the fibres with the non-paramagnetic *N*-ethylmaleimide (2 mM) for 2 h eliminated both active tension and the oriented component of the active fibres. Thus, the oriented component seems to represent active cross-bridges, and its magnitude may be decreased by 20% due to the inactivation of some cross-bridges during labelling.

Although the EPR spectra in Fig. 1 report most directly on orientation, they provide information about the fraction of myosin heads attached to actin. Other work, using conventional EPR to measure orientation[12] (as described here), and saturation transfer EPR to measure motion[13-15], have shown that probes are highly oriented and immobile when myosin is attached to actin (in fibres or myofibrils in rigor, or in actomyosin) but are highly disoriented and mobile (on the μs scale) when myosin is detached from actin (in fibres at long sarcomere lengths or in relaxation, or in purified myosin filaments). Saturation transfer EPR experiments on myofibrils show that most of the probes are as mobile during contraction as in relaxation, suggesting that most are detached[13]. Thus, the most straightforward interpretation of the data in Fig. 1 is that during tension generation, the myosin head spends ~20% of its time attached to actin, and that for most of that time the orientation of the probed region is identical to that in rigor. In principal, we cannot exclude the possibility that some of the disordered probes are attached in either relaxation or tension, but this seems unlikely in view of the previous observations that they have the same degree of disorder and motion as in fibres stretched beyond overlap. The fraction of attached heads in contracting fibres has been estimated using various techniques. Fibre stiffness and X-ray diffraction have suggested, respectively, that roughly 70% and 50% of the heads are attached[16,17]. However, values close to that found here are obtained from calculations based on the ATPase activity rate of isotonically contracting frog fibres (ref. 18 and W. Bialek and R.C., unpublished), and from a study of the motion of a myosin-bound spin label[19].

Information on cross-bridge dynamics has been obtained from measurements of the transients in tension or sarcomere length that follow step changes in muscle length[20,21]. Although there are many possible molecular explanations of these and other transient phenomena, they strongly suggest that the predominant attached states during isometric tension occur early in the power stroke, not at the end[20,23]. Nevertheless, our EPR spectra indicate that the predominant probe orientation is identical with that of rigor, the state which is usually assumed to be at the end of the power stroke. These results suggest, therefore, that states early and late in the power stroke have the same probe orientation, that is, the probed region of myosin does not rotate during the power stroke. This is supported by the observation that stress does not alter the orientation of spin labels on myosin heads in rigor fibres, a result which shows that the angle of the spin label is not linked to the force supported by the fibre[24]. It is possible that the probe is oriented relative to the myosin head at such an angle that myosin rotation does not affect the probe orientation, but this is extremely unlikely in light of the high resolution of the rigor-like component of the contracting fibres; neither the centre of the distribution nor its width differs from that of rigor by more than 1°.

There are many different models that fit the conclusion of this study. One possibility is that the entire myosin head maintains a constant orientation during the power stroke, while some other part of myosin shortens; one example of such a model has been proposed[25]. Alternatively, the probed region of myosin may remain rigidly oriented on actin, while another domain of the myosin head does rotate. There is, as yet, insufficient evidence to exclude either possibility.

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BOOK REVIEWS

Mapping the evolution of biochemistry

Frederic L. Holmes

IN THIS first full-length study of the institutional structures within which the family of sciences known today as "biochemistry" have grown to maturity, Robert Kohler describes the formation, development, and sometimes the decline, of the various administrative organizations within which that field has been practised and taught. Kohler bases his account on an impressive array of unpublished institutional and individual archives; his aim is to reconstruct representative events underlying what he defines as "the changing political map of scientific disciplines".

Beginning with the origins of physiological chemistry in Germany between 1840 and 1900, Kohler recounts how, after a promising start, that field failed to develop independent institutional foundations. The subject was pursued instead within other disciplines such as physiology and pathology. Even though German scientists exerted much of the leadership in biochemical research then and on into the early twentieth century, they failed, according to Kohler, to build a discipline. Equally, in Great Britain physiologists resisted the formation of separate departments of physiological chemistry, even though they regularly hired organic chemists to fill the posts in physiological chemistry. The major exception to this pattern was the Cambridge school of general biochemistry, which Frederick Gowland Hopkins was able to establish in the 1920s with the aid of an outside foundation.

Until the 1930s, Germany and Britain provided most of the leading scientists in biochemistry. The United States remained, with some notable exceptions, a research backwater. Nevertheless Kohler devotes over two-thirds of his book to American biochemistry between 1900 and 1940. His justification for this apparent imbalance is that in the United States biochemists were able to achieve the independence which eluded their counterparts in Europe. Kohler shows in detail how this feat was managed in representative American schools. It was the reform movement in American medical schools at the turn of the century, especially the drive to introduce "scientific" medicine, that provided physiological chemists with their opportunities; and it was, Kohler repeatedly emphasizes, because the biochemists established a "service role" teaching medical students that they were able to command the political and financial backing necessary to maintain departments, to support teaching and research positions, and to find jobs for those they trained. At the same time, most efforts to introduce biochemistry as a broadly-based, biological subject into scientific schools and undergraduate colleges failed for lack of a stable service role.

From Medical Chemistry to Biochemistry: The Making of a Biomedical Discipline. By Robert E. Kohler. Pp.399. ISBN 0-521-24312-2. (Cambridge University Press: 1982.) £22.50, $34.50.

Kohler skilfully uses selective case-histories of prominent medical schools to build up a picture of what he regards as the typical patterns of institutional development. Particular departments which departed from these patterns he treats as "deviant" cases, even though the groups which worked in them included some of the most distinguished and influential leaders in the field. He presents his case-histories in short, well-paced narratives, including lively character sketches of the personalities involved, with entertaining descriptions of the manoeuvres in which they often engaged as "discipline builders" and of the disasters which overcame some of the less capable entrepreneurs.

At his best Kohler writes with a felicitous style; sometimes, however, he resorts to distractingly colloquial language and to deliberately political metaphors, such as "physiologists developed an expansive imperialistic program", and "British physiologists were determined opponents of home rule for physiological chemistry". These phrases are designed to reveal scientists as politically motivated and fundamentally no different from any other self-interest group. Use of such language, however, is a cheap way to make the point. It does so merely by colouring the historical figures with the historian's conception of them, without taking the trouble to demonstrate that these people were not primarily motivated by a commitment to their scientific calling.

Kohler's account, based as it is on extensive archival sources, is convincing. There is reason to be wary, however, about the reliability of some of the factual detail. To take one example, chosen because it is familiar to me, Kohler refers briefly to aspects of the career of Hans Krebs in seven different places in his book. Three of these passages contain mistakes. Kohler says (p.71) that Krebs was given a research position at Sheffield in 1938, whereas the correct date was 1935. He writes (p.326) that Krebs made "a smooth transition from pathology to biochemistry in the mid-1930s"; in fact, Krebs was never a pathologist, and he had been involved in biochemical research since 1926. Finally, in a footnote on p.386, Krebs is described as "one of the first outside Beadle's circle to do biochemical genetics", despite the fact that Krebs was never personally involved in research in that field. These are not trivial errors, for in each case the author is illustrating a general point which depends upon the misinformation in question.

The aim of *From Medicinal Chemistry to Biochemistry* is far more ambitious than simply to map out the institutional setting within which the discipline of biochemistry developed, for Kohler's thesis is

> that biochemists' programmatic conceptions of their discipline were shaped by institutional contexts and relationships, such as channels of recruitment, political alliances, and service roles.

Repeatedly he views "research programmes" as arising from local circumstances, tied to strategies appropriate to success within a particular medical school, chemistry department or other institutional arrangement.

It is certainly true that such local conditions influence the type of science practised within individual organizations, but only in the sense that they define which of the possible niches within the broader research stream a particular local group can attempt to occupy. It is not the local patrons who adjudicate whether the research carried out within their organizations is significant enough to merit recognition; rather it is colleagues pursuing similar problems in other centres — comprising the international community, or "invisible college" of a particular speciality :— who make such judgements. We cannot evaluate the degree to which institutions shape research programmes until we have a thorough grasp of what is being shaped. Kohler touches frequently upon the research areas that the biochemists in his story pursued, but he never penetrates deeply enough into these topics to assess the interplay of influences which directed them.

In Joseph Fruton's masterly *Molecules and Life* (Wiley, 1972) and Marcel

Florkin's more encyclopaedic *A History of Biochemistry* (in *Comprehensive Biochemistry*, Vols 30–33b, Elsevier; 1972–1982) we have two very useful descriptions of the main lines of investigation that have characterized the biochemical family of sciences over the past two centuries. Kohler's work on institutions complements these accounts, but he chooses to dismiss the work of Florkin and Fruton as representative of the allegedly outmoded approach of an earlier generation of "insiders". Without their help, however, and much more besides in the way of detailed studies of specific areas of biochemistry, Kohler's assertions about the shaping force of institutions will remain empty generalizations.

By reconstructing the institutional contexts within which biochemistry developed, Kohler has made progress towards an eventual description of the contours of that subject. A modern scientific discipline is, however, an enormously complex cluster of individual leaders and followers, schools, sub-specialities, literature, methods, concepts, traditions, careers, solved and unsolved problems, evolutionary and revolutionary events, as well as a set of institutional organizations. To understand the interplay of all these factors is a formidable task, the magnitude of which social historians of science have scarcely begun to appreciate. □

Frederic L. Holmes is Chairman of the Section of History of Medicine, Yale University School of Medicine, and President of the History of Science Society.

Programs for seeing

Oliver Braddick

Computer Vision. By Dana H. Ballard and Christopher M. Brown. Pp.523. ISBN 0-13-165316-4. (Prentice-Hall: 1982.) £33.95, $39.95.

BALLARD and Brown intend their book ". . . both as a senior/graduate level text and as a useful reference to those building vision systems". These are admirable and ambitious goals in a field where most books so far have been symposium proceedings or collections of papers, and where the primary literature tends to be a bibliographic jungle of technical reports and conference contributions.

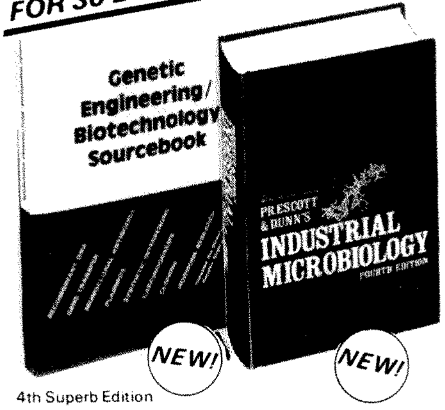
The authors have certainly succeeded in providing a valuable work of reference. The book contains a compendium of the techniques that have been found useful at every level of computer vision systems. If you need an outline of the different possible tesselations of the image plane, the representation of illuminated surfaces in gradient space, chain codes for boundary representation or graph-matching algorithms, these and much more are presented at a sufficiently basic level that they will make the research literature much more accessible to the student or outsider. The frequent use of concrete examples from the programming of vision problems helps to overcome the risk of daunting abstractions and to make clear that the authors' accounts of some general artificial-intelligence issues, such as production systems and semantic nets, do have their relevance to the specific needs of vision.

The goal of making computer programs that obtain knowledge of the world from optical information can be approached from either end. One approach is the search for operations which are effective in extracting from the optical input that information which purely and reliably conveys the significant properties of the world, stripped of the incidental variations of viewpoint, illumination, noisy imaging processes and so on. The other is to realize the richness of prior knowledge that human beings bring to perceptual tasks, and to seek ways in which programs can efficiently use such knowledge to organize the noisy deluge of optical information.

Both approaches are fairly represented in Ballard and Brown's book. Someone planning a vision system might reasonably ask how far they can expect to take "bottom-up" information extracting procedures, and what kinds of otherwise intractable ambiguities or overloads may be dissolved by the "top-down" application of world knowledge. Certainly this is the sort of issue which those interested in human vision hope will be illuminated by computer vision studies. However, the book is less strong on providing this kind of overview. "Top-down" versus "bottom-up" processing may be too over-arching an issue for much resolution of it to be expected, but similar criticisms could be made on a smaller scale. A wealth of techniques is offered, but rather less evaluation of how well they handle various kinds of problem, or consideration of *why* a particular method should be relatively effective or ineffective. The latter is an ambitious demand; but perhaps a field which is still looking for the necessary organizing principles cannot yet sustain a true textbook.

These problems may be a reflection of a more general tension within computer science: is it the body of guidelines to effective practice which underlies a technology, or the body of results about the nature of the world which constitutes a science? Many people are convinced that there is, in the making, a science of how both artificial and biological vision systems can work: Ballard and Brown provide a rare guidebook to trails on the slopes of this unclimbed peak.

Oliver Braddick is Lecturer in Experimental Psychology at the University of Cambridge.



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Yet more population modelling

Robert H. Smith

Modelling Fluctuating Populations. By R.M. Nisbet and W.S.C. Gurney. Pp.379. ISBN 0-471-28058-5. (Wiley: 1982.) £19.50, $47.50.

THE APPEARANCE of yet another book on population modelling written by yet more mathematicians/physicists/engineers with an enthusiasm for ecology is, on a probabilistic basis, unlikely to arouse much interest amongst ecologists. With a few notable exceptions, such texts are mostly regarded as excuses for mathematical self-indulgence and are seldom read by ecologists, apart from those of us unfortunate enough to be asked to review them. However, *Modelling Fluctuating Populations* is exceptional in more than one way, and provided me with an unexpected pleasure; I enjoyed almost all of the book and found every chapter valuable.

Although there have been many books on mathematical ecology since Lotka's seminal contribution *Elements of Physical Biology* (Williams & Wilkins, 1925), few are memorable. May's *Stability and Complexity in Model Ecosystems* (Princeton University Press, 1973) is an exception and must be regarded as one of the most important books in the field; as well as some new insights into the subject matter of the title, the book provided a lucid account of the mathematical techniques used by physicists to analyse equations of motion. The main shortcoming of May's book was the deliberate omission of stochastic models, apart from the use of Kolmogorov's diffusion equation to represent a particular type of environmental variability.

In *Modelling Fluctuating Populations*, Nisbet and Gurney have given a thorough and rigorous account of the techniques available for investigating the dynamic properties of models, both deterministic and stochastic. Just as important, they devote some space to the problem of formulating the most appropriate mathematical representation of a process. Most ecologists will find the mathematics hard to follow, but not impossibly so; and at least the authors do not pretend that difficulties both in manipulation and interpretation do not exist.

The presentation of text and equations is of a very high quality, but unfortunately the same cannot be said of the lettering of the figures, many of which call for the use of hand-lens — in this respect the book is more suitable for the field ecologist than the theoretician. Some of the worst examples are where material has been taken from other publications and photoreduced (e.g. Figs 2.15 and 2.16), but there are many other such instances among the figures drawn especially for the book (e.g. Figs 2.9, 5.8 and 7.13). It is a pity that an otherwise excellent production is marred by such an elementary but important flaw.

Like many before them, Nisbet and Gurney find themselves in deep water when they try to put their ecological models into an evolutionary context. Stability is a property of populations and is not a direct consequence of natural selection, and Nisbet and Gurney are honest enough to invoke group selection explicitly when they suggest why limit cycles might be rare in nature. Leaving aside the difficult question of what conditions are necessary for group selection to be of any importance, the authors have a clear penchant for point equilibria rather than stable limit cycles, and most of their linear approximations require the existence of a stable equilibrium point. However, they do not shirk the task of coping with non-linearity where necessary as evidenced by their interesting treatment of Nicholson's blow-fly in Chapter 8. This chapter, and the other two case-studies, are very worthwhile and in themselves provide adequate justification for the rest of this excellent book. ☐

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The evolution of landscapes

J. Rose

Climatic Geomorphology. By Julius Büdel. Pp.528. Hbk ISBN 0-691-08294-4; pbk ISBN 0-691-08295-2. (Princeton University Press: 1982.) Hbk £40.62, $65; pbk £15.31, $24.50.

OVER the past few decades geomorphology in most English-speaking countries has primarily dealt with the interaction between particular surface processes and the resisting surface materials. For the sake of practicality most of these studies have been concerned with isolated problems, river-channel bedforms or hillslope hydrology for example. The results of this work have been summarized in systematic texts such as Sugden and John's *Glaciers and Landscape* (Edward Arnold, 1976) and Schumm's *Fluvial System* (Wiley, 1977).

Similarly, where English-speaking geomorphologists have investigated the evolution of landscapes through time they have largely adopted an interdisciplinary approach within the framework of the Quaternary; again, these studies have been the subject of significant texts such as Goudie's *Environmental Change* (Oxford University Press, 1977) and Bowen's *Quaternary Geology* (Pergamon, 1978). An explanation for the wider span of landform assemblages and integrated landform development has received little attention other than in Pitty's *Introduction to Geomorphology* (Methuen, 1971), and this has been far from widely understood. *Climatic Geomorphology* by Jülius Büdel, now Emeritus Professor of Geography at Würzburg, Germany is an unashamed attempt to remedy this deficiency.

Büdel's view of geomorphology consists of an investigation of the processes involved, which he calls "active geomorphology"; an appreciation of the role and magnitude of contemporary landscape alteration, which he terms "dynamic geomorphology"; and the global stage upon which the processes take place, to which he gives the title "climato-genetic geomorphology". "Climatic geomorphology" is the foundation for all these approaches and is the thesis of this book.

In many respects *Climatic Geomorphology* is a personal expression of over 50 years' geomorphological experience from all regions of the globe. Although there are some distinctive personal traits, such as a degree of contempt for the isolated studies that have dominated geomorphological research in the English-speaking world, it is far from out of date, with some 38 per cent of the references published in the decade before it went to press, and far from parochial with 19 per cent of the references in the English language. The book is divided into three parts: an introductory section which explains the geomorphic processes, interactions and spheres of operation in a concise but illuminating fashion; a main body of text which reviews the global landscape in terms of morphogenetic regions; and a series of case-studies from selected areas to illustrate the principles so developed. This last section gives particular emphasis to aspects of the central European landscape.

To a considerable extent, Büdel's approach to geomorphology, and hence the rationale behind his book, can be understood in terms of his desire to explain the landscape of central Europe with geomorphologically active Alpine mountains and geomorphologically quiescent incised valleys and upland plains. The mountain and glacial morphogenetic zones he considers to be globally ubiquitous and therefore receive little attention. The incised valleys are the goal of his study of "ectropical" regions and the results of fieldwork in Spitzbergen lead him to believe that bedrock disruption and disaggregation by ground ice beneath river channels provide the mechanism for "excessive valley deepening". Although the argument is clear and well illustrated it is, in my opinion, far from convincing and much of the evidence put forward by Büdel for contemporary *erosion* in his research area is not valid, but is the product of contemporary *deposition* in a region of aggrading ground ice. His quest for an explanation for geologically discordant plateaux takes him to the "peritropical" zones of contemporary etchplain development which he sees as the process for the formation of extensive, low-relief plains. To most of the British geomorphologists interested in cold-climate processes his descriptions of weathering and wash processes in humid tropical regions give a most illuminating explanation for our much-abused and little-understood erosion surfaces.

In many ways, Büdel's views must make geomorphologists in the English-speaking world reassess the results of their single-case, analytical studies. For instance, it is interesting to consider if the approach taken by the contributors to Derbyshire's *Geomorphology and Climate* — published in 1976 (by Wiley) one year before *Climatic Geomorphology* appeared in Germany — gives a greater insight into how landforms and landscapes develop, if it achieves less or if it says the same thing in different ways. For what it is worth, my views are that the recent efforts of English-speaking geomorphologists have contributed much that is of significance to their subject, and that much of Büdel's criticism is unjustified. Equally, however, I believe that without the wisdom shown by Büdel's synthesis many of the individual analytical studies will be wasted.

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Bonding chemistry unravelled

B.F.G. Johnson

Multiple Bonds Between Metal Atoms. By F. Albert Cotton and Richard A. Walton. Pp.466. ISBN 0-471-04686-8. (Wiley: 1982.) $63.20, £39.50.

I ADMIRE few chemists as much as F.A. Cotton. He has had a long and successful career and became one of the few inorganic chemists to achieve international prominence, both through the excellence of his research and because he can always be counted on to provide a clear description and cogent explanation of his chemistry. He is a highly skilled author and together with Dick Walton has written a classic book on multiple bonds between metal atoms. This (in my opinion) is Cotton's favourite topic and it shows.

The book examines the fundamental chemistry of compounds containing metal-metal bonds with bond orders greater than one. The study of such compounds has contributed much to modern inorganic chemistry. Unravelling the bonding problems provided by their structures has provided a wonderful stimulus to researchers, from which a totally new and exciting branch of chemistry has emerged.

"Introduction and Survey", the first of the eight chapters in the book, provides a fascinating insight into the genesis of this area of research and how it continues to progress. Apart from this and a further chapter devoted to physical, spectroscopic and theoretical results, the book deals with the formation of multiple bonds found between metal atoms (rhenium, molybdenum, tungsten and rhodium), and with their synthesis, structure and chemistry. Each chapter has been carefully sub-divided into small packages of information, thereby providing a concise and easily followed account. By so doing those of us concerned with compiling a succinct lecture course or simply locating information relevant to our own research will have few problems. There is a wealth of good diagrams and tables, the literature survey is first class and the overall standard of production is excellent.

Cotton and Walton have provided a clear insight into the chemistry and importance of multiple metal–metal bonds. They have written a valuable and entertaining mix of chemistry, reminiscence and opinion, and have thereby given us an enjoyable, readable book which is a must for all practising inorganic chemists.

B.F.G. Johnson is Reader in Inorganic Chemistry at the University of Cambridge.

Everyman's guide to molecular cloning

Noreen E. Murray

Molecular Cloning: A Laboratory Manual. By T. Maniatis, E.F. Fritsch and J. Sambrook. Pp.545. Pbk ISBN 0-87969-136-0. (Cold Spring Harbor Laboratory: 1982.) $40 (US), $48 (elsewhere).

FOR ten years the Cold Spring Harbor publication *Experiments in Molecular Genetics* by J.H. Miller has been a popular and reliable reference book for those working with *Escherichia coli* and its phages. I had anticipated that the laboratory manual *Molecular Cloning* would serve a similar role for those using *E.coli* as host and its plasmids and phages as vectors for the cloning and analysis of DNA. The long awaited manual is now available and I expect few readers to be disappointed.

The manual emphasizes both the biological systems and biochemical tools available. Each topic is introduced clearly, though concisely, taking care to equip the reader with some understanding of the systems and techniques he may use. Quite elementary information is included and a motivated, and perhaps green-fingered, novice should be able to start from simple experiments and rise to the more sophisticated technology, given, of course, the availability of a battery of good enzymes. Even here advice is offered about the careful use and preservation of enzymes. Although the volume is a laboratory manual it is not lacking in basic theoretical information, is well supplied with references and is amply provided with clear illustrations.

The authors have covered the predicted range of topics including vector–host systems, gel electrophoresis, isolation of mRNA, cDNA cloning, the construction of gene libraries and the detection and analysis of recombinant molecules. The information is supplemented with useful appendices full of recipes and simple biochemical methods. In some areas the manual is up to the minute, particularly in its presentation of a recombination method for screening bacteriophage lambda libraries for specific DNA sequences. In contrast it denies the usefulness of bacteriophage λ as an efficient means of cloning cDNA despite contrary documentation in the literature early in 1981.

One criticism that I would make of this otherwise excellent manual, a criticism that possibly reflects the pressures of a fast-moving field, is the presence of more errors than one usually associates with Cold Spring Harbor publications. Thus a list of around 20 *E.coli* strains includes at least five mistakes. Some of these — for example, *supF* where it should be *supE* — are likely to confuse the beginner who will assume that he himself, and not the manual, is in error. In similar vein, λ*sep6-lac5* is stated to be exceptional in having the *red* and *gam* genes in its right arm; in fact, it is not exceptional and furthermore, since it is *gam*$^+$ and *red*$^-$ (not *red*$^+$) it may be propagated on a *recA*$^-$ host in the absence of both phage and host recombination systems.

Despite such blemishes I strongly recommend this manual. Those working in the area of molecular cloning, or contemplating doing so, may have received their copy already; others should put their name on the waiting list.

Noreen Murray is Reader in the Department of Molecular Biology at the University of Edinburgh.

Nature publishes an annual review of new journals. This year's New Journals Review appeared in the issue of 7 October.

BOOKS RECEIVED

Astronomy

FRICKE, W. and TELEKI, G. (eds). Sun and Planetary System. Proceedings of the 6th European Regional Meeting in Astronomy held October 1981, Yugoslavia. Astrophysics and Space Science Library, Vol.96. Pp.504. ISBN 90-277-1429-0. (Reidel: 1982.) Dfl.150, $65.

FRIEDJUNG, M. and VIOTTI, R. (eds). The Nature of Symbiotic Stars. Proceedings of IAU Colloquium no.70 held August 1981, Observatoire de Haute Provence. Astrophysics and Space Science Library, Vol.95. Pp.302. ISBN 90-277-1422-3. (Reidel: 1982.) Dfl.100, $43.50.

GOUDIS, C. The Orion Complex: A Case Study of Interstellar Matter. Astrophysics and Space Science Library, Vol.90. Pp.475. ISBN 90-277-1298-0. (Reidel: 1982.) Dfl.140, $59.50.

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JASCHEK, C. and HEINTZ, W. (eds). Automated Data Retrieval in Astronomy. Proceedings of the 64th Colloquium of the International Astronomical Union held July 1981, France. Astrophysics and Space Science Library, Vol.97. Pp.326. ISBN 90-277-1435-5. (Reidel: 1982.) Dfl.110, $48.

REES, M.J. and STONEHAM, R.J. (eds). Supernovae: A Survey of Current Research. Proceedings of the NATO Advanced Study Institute held June–July 1981, Cambridge. Pp.580. ISBN 90-277-1442-8. (Reidel: 1982.) Dfl.160, $69.

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HITTINGHAM, M.S. and JACOBSON, A.J. (eds). Intercalation Chemistry.
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Earth Sciences

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ANNOUNCEMENTS

Appointments

Dr Vincenzo Buonassisi has been appointed deputy director of the W. Alton Jones Cell Science Centre.

President Reagan has nominated **Mr Robert Gilkeson, Dr William Miller** and **Dr William Nierenberg** to serve for six-year terms as members of the National Science Board, the policymaking body of the National Science Foundation.

The British Secretary of State for Foreign and Commonwealth Affairs has appointed the **Hon John D. Eccles** as a member of the board of the Commonwealth Development Corporation for three years.

Professor J. Lloyd and **Mr G. Telling** have been appointed as members of the Food Additives and Contaminants Committee. **Dr W. Elstow**, and **Professor P. Turner** have been re-appointed as members of the Committee.

Mr Nigel Lawson, the British Secretary of State for Energy, has appointed six new directors for Britoil: **Mr Michael Kelly, Mr Larry Tindale, Sir Archie Lamb, Mr Ralph Quartano, Sir Alistair Frame,** and **Sir Kenneth Corfield.**

Dr Paul Winson has been made marketing manager for the Oxford Instruments Group, UK.

Techne (Cambridge) Limited have recently appointed a new sales and marketing manager, **Mr M.B.C. Sharp.** Mr Sharp has taken over the complete marketing function embracing both UK and export sales.

Mr Peter James has been appointed managing director of Fisons scientific equipment division's Australian subsidiary Townson & Mercer.

Awards

The Hammer Prize Foundation has awarded its first $100,000 prize for cancer research to **Ronald Levy** of Stanford University, USA and **George Stevenson** of the University of Southampton, UK. They were cited for their work on monoclonal antibodies in the treatment of B-cell leukaemia and lymphoma.

Three industrialists have been elected trustees of Scripps Clinic and Research Foundation, the biomedical research and health care institution in La Jolla, California. These are **David Clare** president of Johnson & Johnson, **Richard Cramer** chairman and chief executive officer of IMED Corporation and **Gordon Anderson** formerly with Baker International, now in private investments.

Professor Vernon Mountcastle, University Professor of Neuroscience at the Johns Hopkins University School of Medicine, has been awarded the Hermann von Helmholtz Award for distinguished research in the cognitive neurosciences. It is the first to be made by the Cognitive Neuroscience Institute of New York.

The Prize Jury of the Novo Foundation of Denmark has decided to award the Novo Prize 1983 to **Professor Christian Crone** of the Panum Institute, University of Copenhagen. The Novo Prize is given as a reward for a major achievement, medical or otherwise, which may benefit the medical science. Professor Crone receives the Prize in recognition of his great contributions towards the understanding of capillary functions.

The winners of the first EPIC award — Education in Partnership with Industry and Commerce — have been announced. Joint first prize winners are the **University of Oxford** in conjunction with **Oxford Instruments Group of Industries** and the **Heriot-Watt University** with **Edinburgh Instrument Limited.** The Heriot-Watt/Edinburgh Instruments partnership won their prize for the development of gas laser and opto-electronic devices. The Oxford winners will use their prize of £25,000 to further develop scanning techniques that do not use X-rays and other applications of superconducting magnets. Runners up for the EPIC award, all of whom received £2,000, were: **Cambridge University** in conjunction with **TAL Chemicals Company** for their development of a fruit coating to control the rate at which fruit ripens; **Liverpool University** and **Rentokil** for the improved manufacture of Rentokil SupaTimber preservatives and the development of a new process for the manufacture of arsenic acid; **UMIST** and **Froude Engineering Ltd** for the production of new dynamometers, devices used in the development of automative engines; and the **University of Wales** plus **Lion Laboratories** for developments in their 15 year long collaboration over the measurement of alcohol in breath samples.

The only fellowship awarded this year by the British Mental Health Foundation has been won by **Dr Susan Wonnacott,** a biochemist at Bath University. The fellowship, worth £40,000 over a 3-year period, will enable Dr Wonnacott to continue her work on nicotinic receptors in the brain, with a view to investigating Alzheimer's disease, a progressive senile dementia which affects large numbers of elderly people. This condition is characterized by a progressive deterioration of mental function, primarily in thought and memory and secondarily in feeling and conduct.

The Bristol-Myers Award for distinguished achievment in nutritional research has been given to **Dr Elsie May Widdowson** of Addenbrooke's Hospital, Cambridge, UK. It is an annual award of $25,000, and this year is the first time that the award will be given to a researcher from the UK.

The Mullard Award has been presented this year to **Oxford Instruments** for their work on advanced superconducting magnets and associated systems, particularly in the area of Nuclear Magnetic Resonance.

A project to develop credit transfer and improve advice on courses available to students has been launched by the Department of Education and Science. A contract, worth nearly £900,000 over three years, has been awarded to the **Open University** to develop and implement the piloting of a computerised Educational Counselling and Credit Transfer Information Service (ECCTIS) in the South West of England.

The Foulkes Foundation, a registered charity which aims to further medical research, has announced the winners of the Foulkes Foundation Fellowship. The fellowship gives financial support to recently qualified science graduates with research experience who want to study medicine, and medical graduates who want to take a science degree. The successful candidates for 1982 are: **Dr D.S. Blanchard, Dr J.P. Burrows, Dr D.A. Gray, Dr P.M. Matthews, Dr R. Silcock, Mr K.A. Steer, Dr C.-K.V. Tse** and **Dr E. Walker.** Entries for the 1983 awards are invited. Application forms will be available from the Fellowship Registrar in January 1983 and applicants are asked to enclose a stamped, addressed envelope.

Entries are invited for the Prince of Wales Award for Industrial Innovation and Production. The Award was first inspired by His Royal Highness in 1980 and aims to identify promising new products or processes and encourage the inventor or entrepreneur to get them into production and onto the market as soon as possible. It is being organized by The Engineering Council. The closing date for receipt of entries is 28 February 1983. Entry forms and further details may be obtained from: The Engineering Council, Canberra House, 10–16 Maltravers·Street, London WC2R 3ER, UK.

ICI has made an endowment of £10,000 to a trust fund which will be administered by The Royal Society of Chemistry, to organise a biennial symposium on industrial chemistry in memory of Dr Alfred Spinks, CBE.

The Ciba Foundation is establishing a bursary scheme linked to the Ciba Foundation symposia. This scheme will enable young scientists to attend Ciba Foundation symposia and subsequently spend a period in the laboratory of one of the major participants. The bursars will be selected from laboratories or institutes not represented at the symposium. The successful bursar's travel, accommodation and other expenses will be met by the award.

Two National Institute on Ageing teaching nursing home grants, totalling $7.43 million over five years, have been made available to support research on geriatric health problems in a nursing home setting. The National Institute on Ageing is part of the US Public Health Service. These are the first grants of their kind to be proposed.

Nominations are invited for the Marconi International Fellowship. Nominees should be individuals whose work in the fields of communication science and technology exemplifies the technical creativity and concern for human welfare of Guglielmo Marconi. The deadline for nominations is 15 April 1983. Final selection of the recipient will be made in October 1983 and the successful candidate will receive £35,000. The Fellowship Selection Committee will give special attention to the identification of emerging fields of communications science and technology. Nominators are asked to specify why they believe that the nominee has made innovative contributions to a field of research or technology of growing future significance.

British Gas has set up a Teaching Fellowship at Cambridge University attached jointly to the Engineering and Chemical Engineering Departments. **Mr. N.K. Macfadyen** has been appointed as its first holder.

Meetings

10-12 January, **The Brain, Biochemistry and Behaviour,** Florida (Ms M. Hack, 355 St Paul's Avenue, Staten Island, NY 10304, USA).

16-22 January, **International Seminar on Human Waste Management,** Bangkok, (Ms T. Liamzon, Seminar Co-ordinator, PO Box 24-130, Bangkok 10240, Thailand).

20 January, **Innovation Conference,** Manchester (Mr J. Jackson, Shirley Institute, Manchester M20 8RX, UK).

January, **Conference on the Palaeo-environment of East Asia,** Hong Kong (R.O. Whyte, University of Hong Kong, Centre of Asian Studies, Pokfulam Road, Hong Kong).

6-10 February, **Third Annual Congress for Recombinant DNA Research,** Philadelphia (Mr E. Ruffing, Scherago Associates, 1515 Broadway, Tenth Floor, New York, NY 10036, USA).

6-10 February, **Second Annual Congress for Hybridoma Research,** Philadelphia (Mr E. Ruffing, Scherago Associates, 1515 Broadway, Tenth Floor, New York, NY 10036, USA).

6-10 February, **"Biotech '83",** Philadelphia (Mr E. Ruffing, Scherago Associates, 1515 Broadway, Tenth Floor, New York, NY 10036, USA).

7-11 February, **Optical Methods in Engineering Metrology,** Colchester (The Liaison Officer, Laser Workshop Courses, University of Essex, Colchester C04 3SQ, Essex, UK).

7-11 February, **Course in Gear Design,** Sheffield (Dr H.K. Kohler, Director for Engineering and Design Courses, Department of Mechanical Engineering, University of Sheffield, Mappin Street, Sheffield S1 3JD, UK).

13-15 March, **First International Risk Seminar,** London (Ms D.E. Jarvis, PO Box 62, Beaconsfield, Bucks HP9 2NY, UK).

4-5 April, **Wind/Solar Energy Conference,** Kansas City (G.H. Stickney, College of Engineering, University of Missouri-Columbia, Columbia, MO 65211, USA).

5-8 April, **Electroanalysis in Biomedical, Environmental and Industrial Sciences,** Cardiff (Dr J.D.R. Thomas, Applied Chemistry Department, Redwood Building, UWIST, Cardiff CF1 3NU, UK).

9-11 April, **Interfacial Kinetics in Solution,** Hull (Professor D.H. Everett, Department of Physical Chemistry, School of Chemistry, Cantock's Close, Bristol BS8 1TS, UK).

11 April, **Opioids: Past, Present and Future,** Cambridge (Dr M.B. Tyers, Neuropharmacology Department, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex UB6 0HE, UK).

11-14 April, **International Conference on Combustion in Engineering,** Oxford (Institute of Mechanical Engineers, 1 Birdcage Walk, Westminster, London SW1H 9JJ, UK).

11-14 April, **7th UK Geographical Assembly,** Leicester (M.A. Khan, Department of Geology, University of Leicester, University Road, Leicester LE1 7RH, UK).

3 May, **International Symposium on Crop Protection,** Gent (The Secretary, Faculteit van de Landbouwwetenschappen, Coupure Links 533, B-9000 Gent, Belgium).

3-6 May, **Canadian Symposium on Remote Sensing,** Montreal (R. Desjardins, Department de Geographie, UQAM, BP 8888, Succ A, Montreal H3C 3P8, Canada).

5 May, **Energy Saving in the Chemical Industry,** London (Rubber and Plastics Research Association, Shawbury, Shrewsbury, Shropshire SY4 4NR, UK).

8-13 May, **Prevention of Occupational Accidents and Diseases,** Ottawa-Hull (The Xth World Congress on the Prevention of Occupational Accidents and Diseases, c/o Canadian Organising Committee, 500-300 Slater Street, Ottawa, Ontario, Canada K1P 6A6).

9-12 May, **International Conference and the Third Annual Meeting of the Electrophoresis Society,** Tokyo (Secretariat Electrophoresis '83, c/o Dr Nobuya Hashimoto, Department of Internal Medicine, The Jikei University School of Medicine, 3-25-8, Nisishimbashi, Minato-ku, Tokyo 105, Japan).

10-12 May, **Progress in Perinatal Medicine** Florence (P.G. Crosignani, IVth Department of Obstetrics and Gynaecology of the University of Milan, Via M. Melloni, 52-Milan, Italy).

10-21 May, **Course on Measurement, Analysis and Control of Noise,** Sheffield (Dr H.K. Kohler, Director for Engineering and Design Courses, Department of Mechanical Engineering, University of Sheffield, Mappin Street, Sheffield S1 3JD, UK).

16-20 May, **International Conference on Radioactive Waste Management,** Seattle (International Atomic Energy Agency, Wagramerstrasse 5, PO Box 100, A-1400 Vienna, Austria).

17-18 May, **Heat and Fluid Flow in Nuclear and Process Plant Safety,** London (The Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London SW1H 9JJ, UK).

17-20 May, **Automated Manufacturing Exhibition and Conference,** Birmingham (Clapp and Poliak Europe Ltd, 232 Acton Lane, London W4 5DL, UK).

17-20 May, **Conference and Exhibition of Lasers and Electro-Optics (CLEO '83),** Baltimore (Publicity Chairman, CLEO '83, Tiemo von Zweck, Laser Power Optics, 11211 Sorrento Valley Road, San Diego, California, USA).

23-27 May, **International Conference on Graphite Intercalation Compounds,** Pont-a-Mousson, France (Dr Daniel Guerard, Laboratoire de Chimie Minerale Appliquee, Université de Nancy 1, BP 239, 54 506 Vandoeuvre les Nancy, France).

24-26 May **International Conference on Road Vehicle Handling,** Nuneaton (The Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London SW1H 9JJ, UK).

24-26 May, **International Conference on Ecology and Environmental Quality** Jerusalem (Professor H. Shuval, Chairman, Israel Ecological Society, Hebrew University-Hadassah Medical School, PO Box 1172, Jerusalem, Israel).

24-26 May, **Energy Storage,** Stratford Upon-Avon (Conference Organiser Energy Storage, BHRA Fluid Engineering Cranfield, Bedford MK43 0AJ, UK).

25 May, **The Ciba Foundation 34th Annua Lecture,** London (Miss J. Holman, The Ciba Foundation, 41 Portland Place London W1N 4BN, UK).

31 May-1 June, **Course on Water Supply and Sanitation for Developing Countries** Ottowa (Dr R. Droste, Water Sanitation Short Course, Department of Civi Engineering, University of Ottawa Ottawa, Ontario, Canada K1N 9B4).